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ANALYSIS OF CURRENT PROFILES BY A STUDY OF PYCNOMERIC DISTORTION AND IDENTIFYING PROPERTIES

By

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It has, of course, always been recognized that one of the greatest uncertainties of dynamic oceanographic calculations is that which results from the necessity of selecting without definite evidence a surface of assumed zero motion to which the calculated relative velocities can be referred for evaluation in absolute terms. In the still most prevalent practice this uncertainty is further increased by the arbitrary assumption of a horizontal zero level, although it is *a priori* highly improbable that the true velocities should be so distributed in all verticals within any ocean area as to obtain zero values at the same absolute depth. The need for abandoning this arbitrary use of a fixed horizontal datum level in favor of a reasonably selected probable current boundary of variable depth has recently been so well stated by Wüst (1936, pp. 52–54) and by Dietrich (1937a, b) that no further discussion seems necessary on these pages. The argument has finally gained further strength from Rossby's theoretical demonstration of the probable occurrence of fairly strong gradient flow near bottom even in very great depths associated with the non-permanent features of ocean currents (Rossby, 1938).

In their search for a true current boundary of variable depth, the German investigators have emphasized the theoretical value and importance of the oxygen minimum distribution as an index to the quasihorizontal movements of the water. In recognition of the isopycnic character of liquid flow, it seemed natural to the present writer also to attempt to locate the current boundaries with reference to the vertical distribution of densities; and when

the conclusions reached from this line of approach, in the manner to be described in the following, are to a large extent found to be in perfect agreement with the results of Dietrich and Wüst this may perhaps be considered a most encouraging indication of the fundamental soundness of the method.

While comparing the presentations of the current situation south of the Newfoundland Bank obtained by dynamic topographies referred to a horizontal datum level and by the isopycnic distributions of the identifying properties (Parr, 1938a), the writer became interested in the possibility of finding evidence of the location of the true current boundaries by a study of the variations in the thickness of the density layers* through vertical profiles laid at right angles to the main direction of flow. In the following this method of attempting to discover the true current boundaries will be illustrated by application to two profiles across the Gulf Stream system, one from Cape Cod to Bermuda, and one off Cape Hatteras; and it might also be mentioned that the first experimental application of the method to several of the profiles in the region of Newfoundland Bank analyzed in other ways by Parr (1938a) gave equally as encouraging results as those to be described herein.

GENERAL REMARKS UPON THE METHOD AND DESCRIPTION OF THE PRELIMINARY PREPARATION OF THE DATA

The method to be suggested in these pages rests upon the assumption that the pycnomeric layers of a current will generally have a tendency to bank themselves to the right of the flow (on the Northern Hemisphere) due to a slight axial velocity downstream in excess of gradient values (Rossby, 1937).

Since currents and counter-currents can, of course, exist side by side in the same pycnomeric layer, it is in this connection necessary to distinguish between lateral current boundaries traversing the isopycnals between adjacent currents in opposite directions within the same pycnomeres, and basal current boundaries separating pycnomerically superimposed movements. While, on the principle of isopycnic flow (Rossby, 1936, Parr, 1938), the basal boundaries should serve to distinguish separate current systems (see also Dietrich, 1937), this is not *a priori* true of the lateral boundaries, which in the presence of eddy circulation may merely serve to separate opposite directions of motion within the same system. In such a system of eddies rotating in harmony with each other, the boundaries of motion will obviously overlap (alternate with) the boundaries of the eddies themselves, since the direction of motion is the same in the adjacent halves of two con-

* Which for the sake of convenience we shall here designate as the pycnomeres, that is, the segments of the vertical column of water confined between two isopycnic surfaces.

tiguous eddies rotating in harmony with each other. These adjacent halves of two eddies rotating in harmony and therefore in opposite directions will consequently belong to the same area of motion in a profile differentiated only with reference to direction of flow, and the boundaries of the *areas of motion* must coincide with the *centers* of these eddies, and not with their own *peripheral boundaries.*

If the assumption is correct that the layers should thicken to the right, and longitudinal flow is isopycnic, a comparison of the increase in depth per unit increase in density $\left(\frac{\Delta D}{\Delta \sigma_t}\right)$ plotted against potential densities *in situ* (σ_t)* for adjacent station verticals in the same vertical section normal to the axis of the current system will show us, by the intercepts of these curves, the isopycnic surfaces along which no increase in thickness, that is, no distortion, occurs in either direction and above and below which such distortions take place. This isopycnic sheet of uniform thickness should then represent a *possible* boundary layer of zero motion since it gives no indication by distortion of being moved in either direction at right angles to our profile.†

This is of course not the same as to say that the uniform thickness of a pycnomere gives *positive* evidence that it is *not* in motion. On the contrary we shall in both of our examples be led to the assumption that certain pycnomeres of uniform thickness are actually moving in the direction of the main flow at the right side of the main current. The proposed method is not direct and absolute, but operates only indirectly by a process of elimination on the assumption that the pycnomeres which *do* show distortion are *not* likely to be static, and that in our search for the probable true current boundaries we may therefore limit our choice to the comparatively few surfaces in which no pycnomeric distortion is apparent, making our final selection with the fullest possible consideration of as many other factors as may have a bearing upon the problem.

The curves given in Figure 88, representing a profile from Cape Cod to Bermuda (above), and a profile across the Gulf Stream off Cape Hatteras

* Although not a strictly correct expression for potential density *in situ* without adiabatic correction of the temperature *in situ*, σ_t has been considered sufficiently accurate for present purposes.

† Since $\frac{\Delta D}{\Delta \sigma_t}$ is simply (in first approximation) the inverse of the expression for vertical stability, our reasoning incidentally leads mathematically to the interesting suggestion that current divides should follow isopycnal surfaces of constant vertical stability. Since constant vertical stability in turbulent flow under otherwise equal circumstances indicates constant horizontal friction (see Parr, 1936; Rossby, 1936), this purely arithmetical result may under otherwise equal circumstances also have a reasonable explanation in fact.

(below),* are obtained simply by dividing the difference in depth between consecutive observations at each station by the difference in density (σ_t) between the same observations, and plotting the result against the linear average of the two densities used in the determination of each value. Figure 88 gives only the lower portions of the entire curves, containing most of the information with which we are here concerned. The complete curves are given in Figure 89 with the values for $\frac{\Delta D}{\Delta \sigma_t}$ drawn on a logarithmic scale for no other reason than the purely mechanical one that it proves absolutely impossible to give a readable picture of these complete curves by the use of a natural scale.

It will be noticed from these figures that nearly all station curves throughout the entire profiles, and absolutely all curves in the region of the main current, have intercepts or contact points with each other at an almost constant density value for all station intervals. Exactly similar general pictures were also obtained for the profiles off Newfoundland Bank on which the method was first tried out.† This finding that the indicated basal current boundary followed a virtually constant isopycnic surface through any transverse profile is patently in excellent agreement with reasonable expectations from the principle of generally isopycnic flow.

When we recall the fact that our curves of $\frac{\Delta D}{\Delta \sigma_t}$ are simply the inverse of the vertical stability curves, we further notice that the basal current boundaries indicated in Figure 88 coincide with a pycnomere which at all stations on the right side (low station numbers above, high station numbers below in Figure 88) of the main current, and also through the greater part of the left portions of the two profiles, is characterized by greater vertical stability than that of the surrounding pycnomeres both above and below.

From Figure 88 we may finally also notice that the relationship between adjacent station curves above and below the indicated basal boundaries are generally the reverse, that is, the basal boundary is identified with a true intercept between these curves. But in the station intervals at the extreme right the boundary is, on the other hand, only identified with contact points

* For the original data see Bulletin Hydrographique 1932. Cons. Perm. Int. Expl. Mer. Copenhagen 1933.

† In the region of the Newfoundland Bank, the general absence of significant distortion occurred around σ_t 27.4–27.5. In the Cape Cod to Bermuda profile, it occurs around σ_t 27.3–27.4, and off Cape Hatteras usually around σ_t 27.2–27.3. Before this apparent trend for the boundary to descend gradually to slightly higher density values (deeper isopycnals) downstream has been abundantly confirmed by repeated applications of the pycnomeric distortion method, one must obviously refrain from drawing any definite conclusions. But if this trend should be confirmed, and not be shown to be merely accidental, it obviously has a considerable theoretical interest.

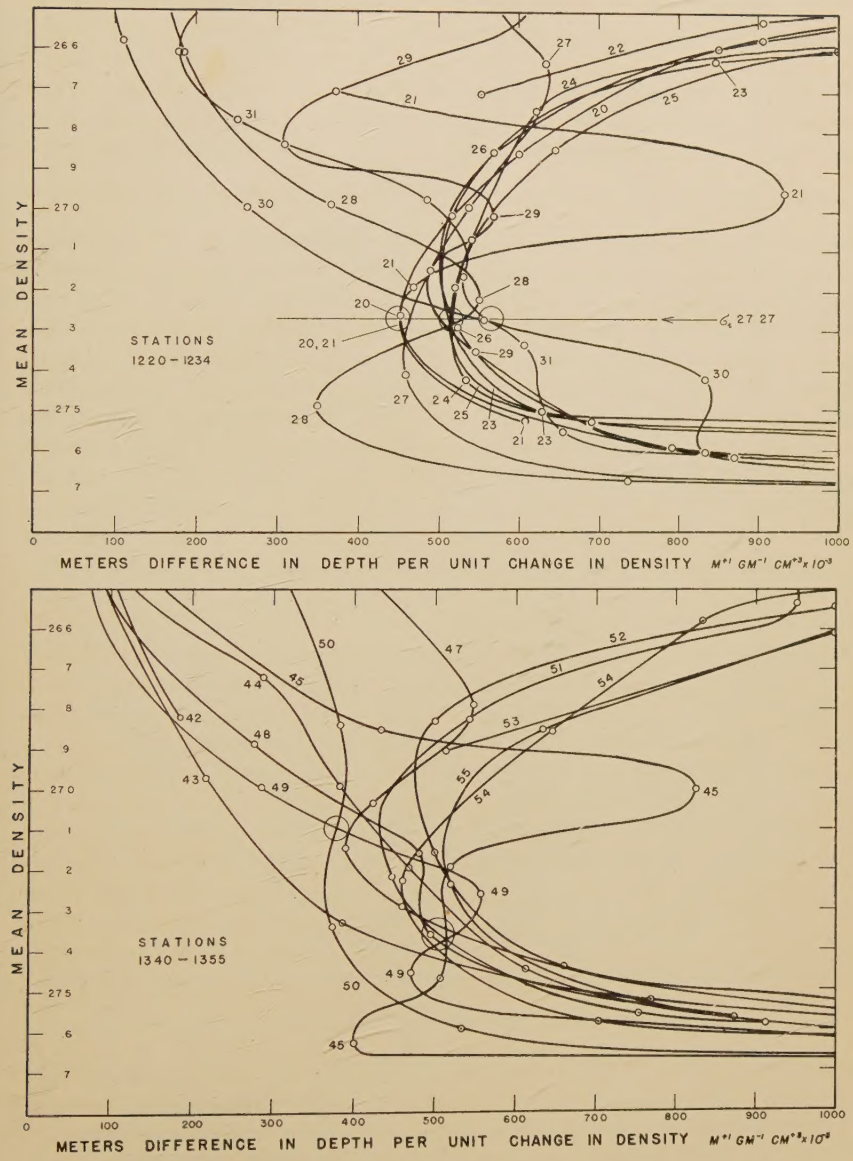

Figure 88. Increase in depth (natural scale) per unit increase in density plotted against density *in situ* (σ_t) for Cape Hatteras profile, stations 1220–34 (*above*), and for Cape Cod–Bermuda profile, stations 1340–55 (*below*).

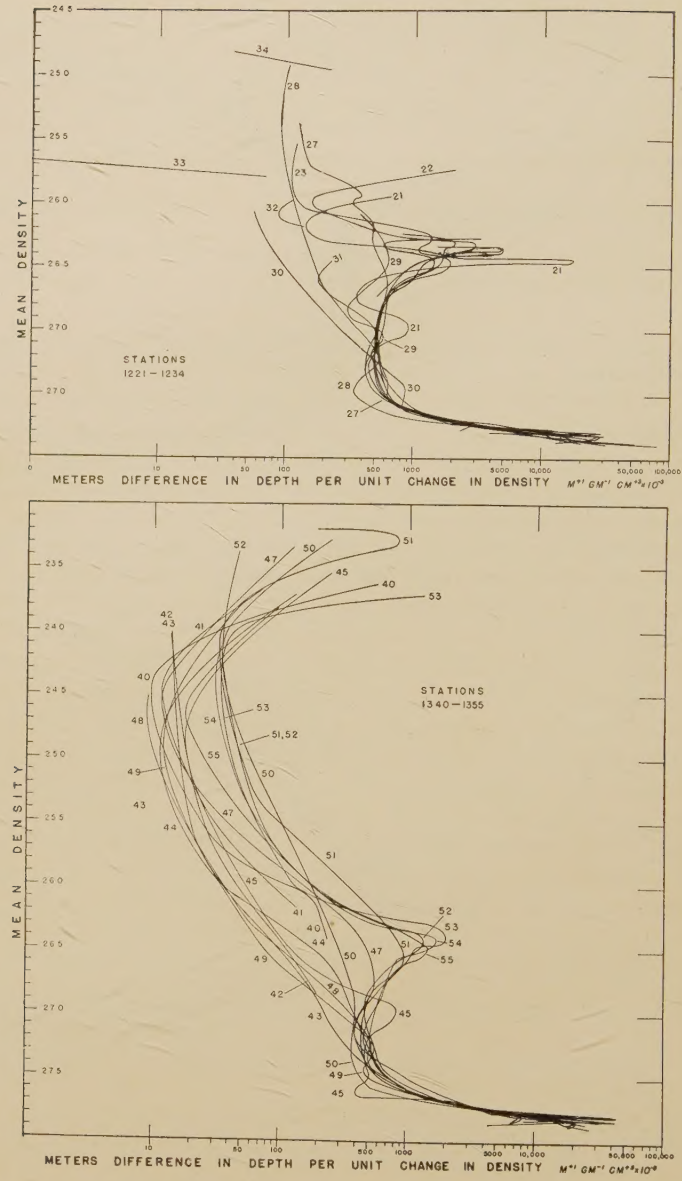

Figure 89. Increase in depth per unit increase in density plotted on a logarithmic scale against density *in situ* (σ_t) for Cape Hatteras profile, stations 1220–34 (*above*) and for Cape Cod–Bermuda profile, stations 1340–55 (*below*).

between the adjacent station curves. That is to say, the station curve having higher values of $\frac{\Delta D}{\Delta \sigma_t}$ above the indicated boundary is reduced to values equal to those of the adjacent stations at the boundary itself, but again shows *higher* instead of lower values below, so that we find an increasing pycnomeric distortion below in the same direction as the distortion above. It seems probable that this equalization of the $\frac{\Delta D}{\Delta \sigma_t}$ values at adjacent stations on the right side of the illustrated profiles is simply a special feature of the general equalization of characteristics brought about by a very active lateral circulation along this particular surface of high vertical stability, which seems to become the true basal boundary surface at the left, but which does *not* separate layers of opposite directions of motion at the right. Since motion in the direction of the main current at the right thus seems to descend without actual interruption to much deeper isopycnals than that followed by the indicated current boundary at the left, we must therefore assume that the true current boundary itself also descends to these deeper isopycnals beyond the region of the main current on the right-hand side of the flow.

Apart from these deviations observed at the extreme right, we also notice in Figure 88 several instances of double intercepts between adjacent station curves, that is, of a second intercept occurring at more variable densities, but always above the general intercept at a nearly constant density value for all station intervals, which we have taken to indicate the true basal boundary of the upper current system. Most of these secondary intercepts above the indicated boundary are due to very minor undulations of the separate station curves, and it is obvious that if the actual curves should approach to fairly uniform values over a short density range immediately above the indicated current boundary, the sources of error in our calculation of the apparent curves would tend to introduce apparent double intercepts where none actually exist. These double intercepts may therefore in part be due to the inaccuracies of the method, but it is also possible that they may have some actual dynamic significance.

Our last paragraph introduces the question of the accuracy with which the $\frac{\Delta D}{\Delta \sigma_t}$ curves can be determined. It is obvious that the accuracy must decrease with a decrease of the absolute differences either in depth or in density between the two observations combined in each determination. Near the free surface the differences in depth are slight and the distorting effect of purely temporary influences from above may be very great. We may therefore take it for granted that a comparison between the upper portions of the station curves very near the free surface would not be of any great signifi-

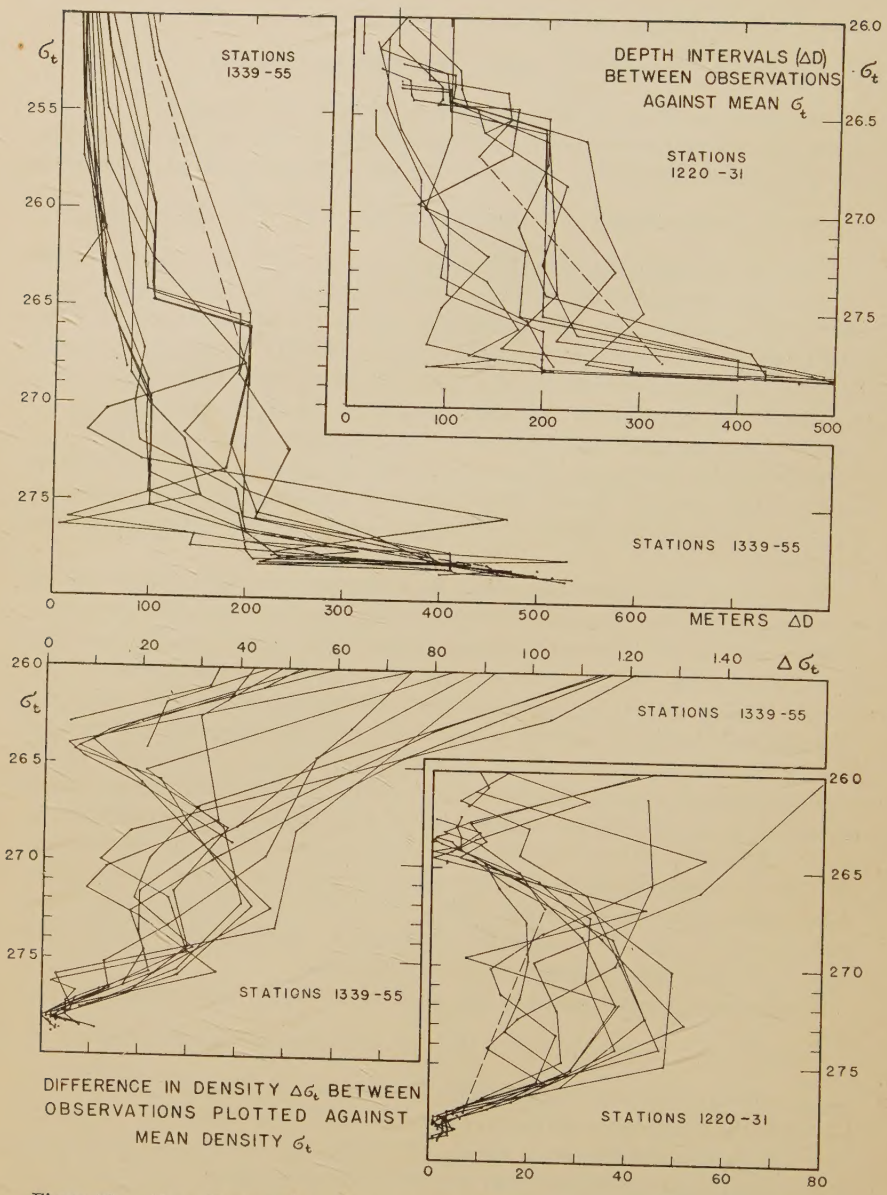

Figure 90. *Above:* Variations in the depth interval between observations plotted against density *in situ* (σ_t). *Below:* Variations in the absolute density difference between observations plotted against density *in situ* (σ_t).

cance. In great depths, on the other hand, the vertical variations in density from one observation to the next become so slight (within 0–5, generally only 0–4, in the second decimal place in the customary notation of σ_t) as to be only very low multiples of the probable errors of determination, if not even less than this error. For the deepest layers our comparisons therefore also become entirely unreliable, and when we reach the conclusion that the true basal boundary on the extreme right descends from its intermediate location at the left to surfaces of very high density, our method does not enable us to determine its precise continuation at the right in terms of isopycnic surfaces, and we have to revert to the old procedure of using a horizontal datum level for the parts of the profiles to which this conclusion applies (see page 285).

From the preceding considerations it is clear that our evidence derived from a consideration of the $\frac{\Delta D}{\Delta \sigma_t}$ curves will be of greatest accuracy and significance where both the density differences and the differences in depth between observations have a fairly high value. It is therefore very reassuring to note from Figure 90 that both ΔD and $\Delta \sigma_t$ between observations are of relatively great absolute magnitudes around the densities with which we have identified our indicated current boundary. With reference to the density differences between observations, there is indeed a tendency for these to develop an intermediate maximum value around σ_t 27.2–27.4, of 0.12–0.50, with an average around 0.30, in the customary notations for σ_t; while the depth intervals vary from 100 to 300 meters. We thus obtain our indications of a basal current boundary in the density zone in which our determinations should have a maximum accuracy and significance.

As the final step in the preparation of the data the curves for relative salinities against σ_t have been drawn in accordance with the procedure previously described (Parr, 1938b). These curves, which are not illustrated, form the basis for the determination of the relative salinity profiles used in the following considerations.

PROFILE ANALYSIS

Having prepared the data for the single stations in the manner described in the preceding, we may now turn our attention to the study of the entire profiles, beginning again with a description of the further procedure.

PROCEDURE USED FOR THE DEVELOPMENT OF INDICATED CURRENT PROFILES

In Figure 92 all details of procedure are indicated in a manner which will, of course, be entirely superfluous by a routine use of the method herein suggested. The information drawn upon comes under two separate headings:

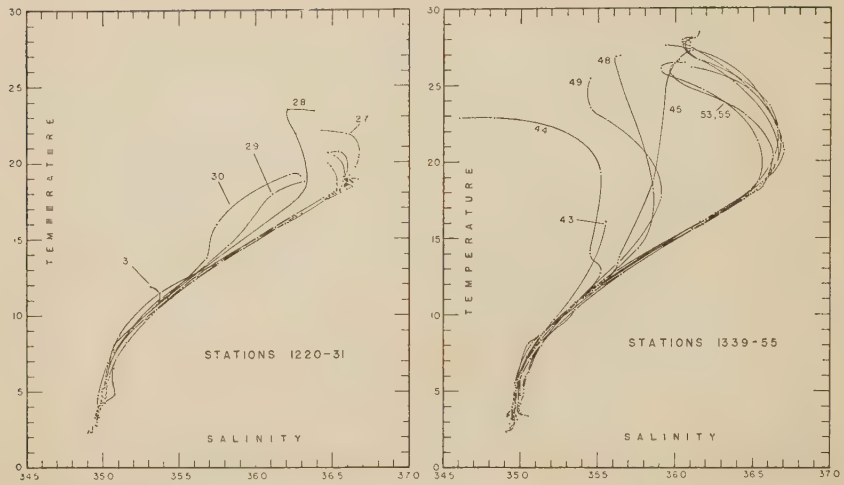

Figure 91. Temperature-Salinity correlation curves in the Cape Hatteras profile (*left*) and in the Cape Cod–Bermuda profile (*right*).

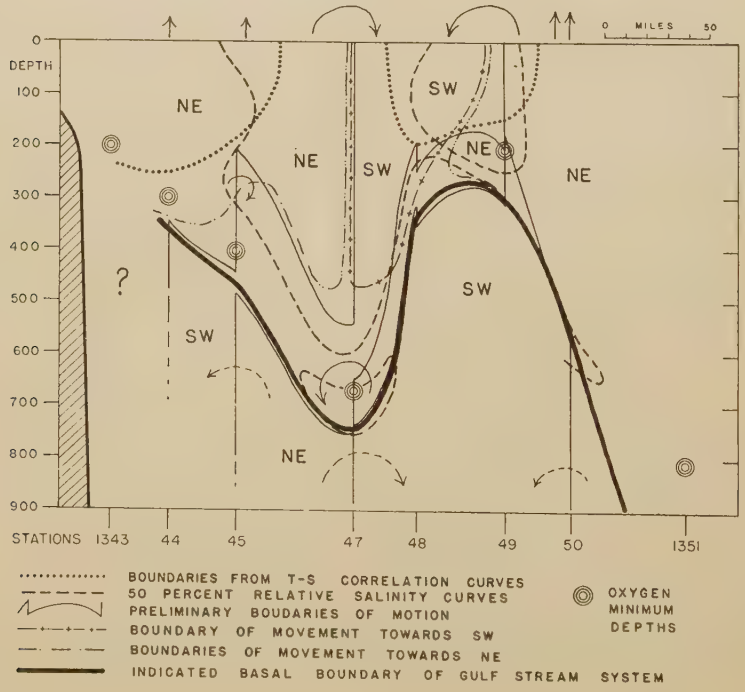

Figure 92. Analysis of the Cape Cod–Bermuda profile. See text.

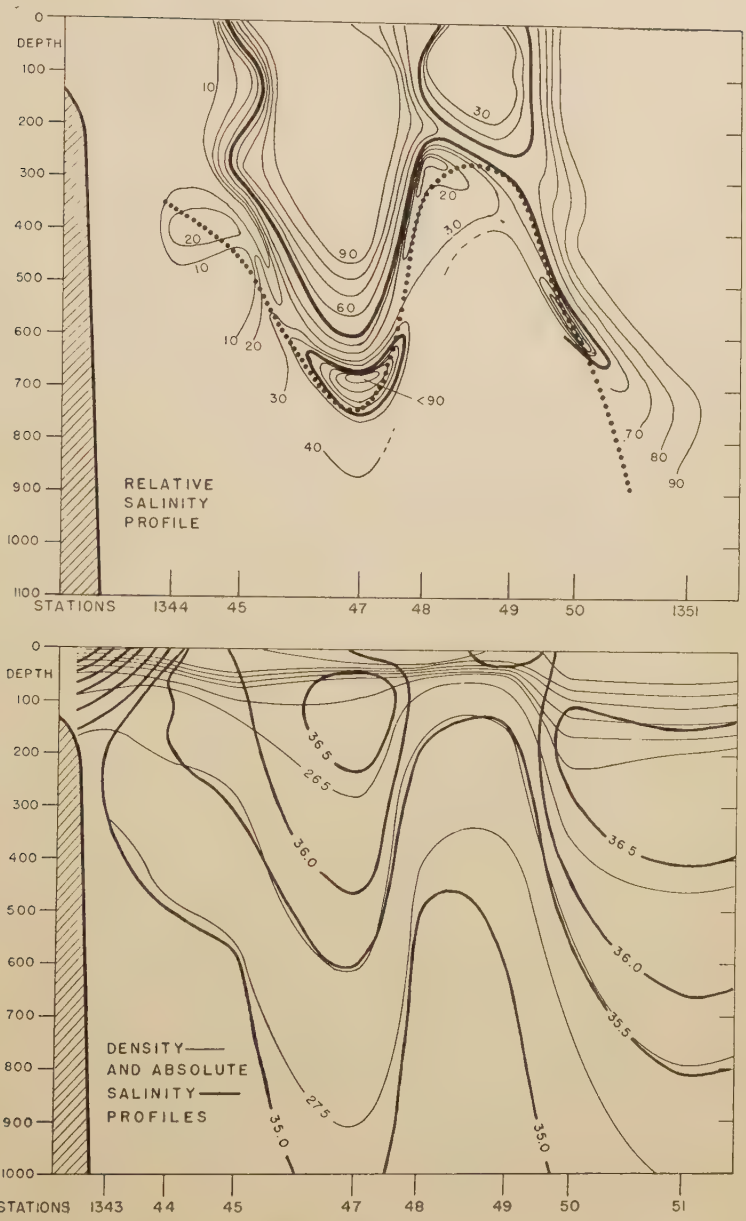

Figure 93. *Above:* Relative salinity distribution in the Cape Cod–Bermuda profile. Heavy contour line for 50% relative salinity. Light lines for every 10%. Dotted curve represents indicated basal current boundary. *Below:* Density and absolute salinity distributions in the Cape Cod–Bermuda profile.

(1) The identity of the water masses. To establish the identities of the water masses, we have first determined the depth of the T–S values at which the deviating upper portion of the T–S correlation curves at the stations in which waters foreign to the Gulf Stream are present seem to join the pattern of the rest of the curves (see Figure 91). By this method we do, of

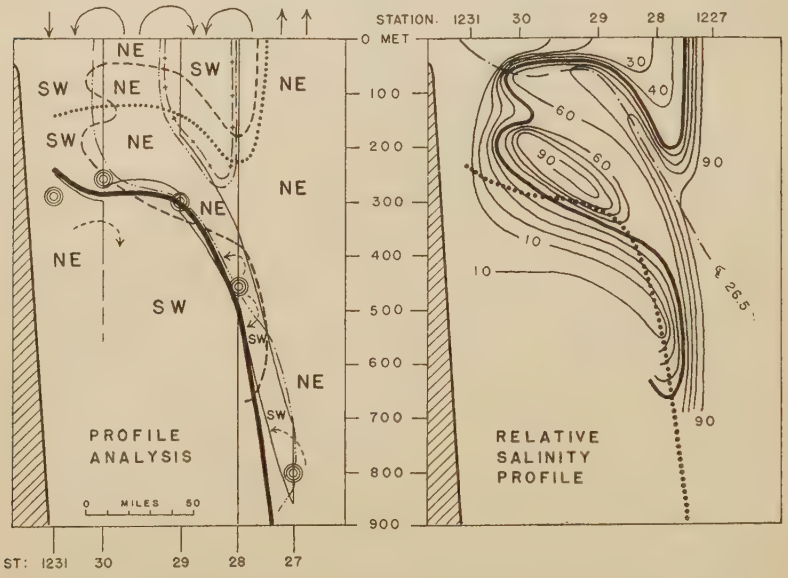

Figure 94. *Left:* Analysis of Cape Hatteras profile. For explanation of symbols see figure 92 and the text. *Right:* Relative salinity distribution in Cape Hatteras profile. See legend of figure 93. In and below the isopycnal surface of $\sigma_t = 26.5$ minimum isopycnic values of salinity occurred at station 1231 throughout the column; while relative salinities above the isopycnal of $\sigma_t = 26.5$ are calculated from minimum values obtained at station 1230, since the upper isopycnals do not extend to station 1231.

course, only get the roughest qualitative differentiation of the water masses in the layers in which the absolute differences are very large and conspicuous at a superficial glance. The depths thus determined have been connected by heavy dotted lines.

To evaluate the finer distinctions, particularly in the deeper layers where the total differences are too slight to be analyzed by the T–S correlation curves alone, we have then also entered in lighter broken lines the relative isohalines of 50% relative salinity obtained from the vertical relative salinity curves mentioned on page 277.

(2) Distribution of motion. From the $\frac{\Delta D}{\Delta \sigma_t}$ curves given in Figure 88 we have determined the σ_t values for the intercept between these curves for ad-

jacent stations, and have entered in light solid lines the course of the corresponding isopycnals between the two station verticals, as a first indication of the presumable boundaries between movements in opposite directions above and below. The presumable directions of motion above and below have been entered according to the direction of distortion shown by the relationship between the two adjacent station curves on the assumption that

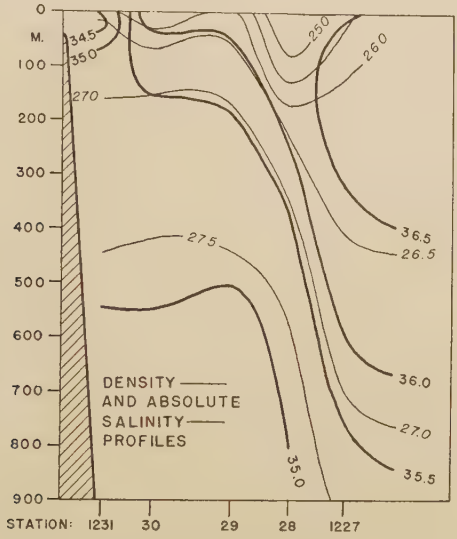

Figure 95. Density and absolute salinity distributions in Cape Hatteras profile.

the thickening of the pycnomeric sheet indicated by higher values of $\left(\frac{\Delta D}{\Delta \sigma_t}\right)$ must occur on the right side of the flow.

When a station is in opposite relations to its two nearest stations on either side with reference to indicated directions of flow, a straight vertical boundary of motion has been drawn at the location of such a station through the depth interval over which such opposite trends are indicated.

Through this procedure we have now divided our profile into areas of indicated movements in opposite directions separated from each other laterally by straight vertical lines, and above and below by undulating isopycnals. It will be noticed from Figures 92 and 94 that even in this discontinuous presentation of separate areas of motion (shown in light solid lines) the lower boundaries approach a confluent pattern in a most encouraging manner even in the very complicated profile from Cape Cod to Bermuda (Figure 92). Nevertheless a proper method of smoothing must be introduced and described.

In this smoothing we have as our main source of freedom the fact that an indicated thickening of pycnomeres at one station compared with the nearest adjacent station merely implies that *greater* thickening has occurred at the former point of observation than at the latter. That is to say, the thickening of the pycnomere at one station as compared with its thickness at the next station gives us no guarantee that this pycnomere is moving in one single direction throughout the station interval concerned. On the contrary, a lateral current boundary may very well be situated between the two stations, so that the pycnomeres should exhibit the effects of thickening due to motion at *both* ends of the station interval, or in other words so that thickening has actually occurred *both to the right and to the left*, indicating movements in opposite directions within the station interval. The only rule which has to be followed is therefore simply that the interpolation of a current boundary within a station interval must be so carried out as to indicate that the *major* motion within the interval must be in the direction shown by a comparison of the thickness of the pycnomeres at the two stations considered, while it is perfectly permissable to suggest a *minor* motion in the opposite direction at the opposite end of the station interval, when other considerations make such a suggestion seem reasonable. On this basis we may therefore move the vertical boundaries away from the exact, but in this connection entirely haphazard, positions of our stations towards the corresponding inflection point of the isopycnals in reasonably drawn density profiles (Figures 93 and 95). This will indirectly also result in a freedom of smoothing the upper and lower boundaries.

Smoothed current boundaries have thus been entered in Figures 92 and 94, with a heavy solid line indicating the probable basal boundary of the entire Gulf Stream system in these profiles.

Having thus arrived at a reasonable analysis of our profiles with reference to directions of motion, we still have to assign these directions of motion to reasonable circulation patterns, and we now have recourse to the differentiation of the water masses indicated by our dotted and broken lines, and shown in greater detail in the relative salinity profiles (Figures 93 and 94). On this point we may be governed by the simplifying assumption that when eddies are indicated each eddy will usually (not necessarily always) rotate without significant change in the identity of its water masses along its closed trajectories. That is to say, each type of water must as a rule form an eddy of its own, and when an area of motion in one direction is divided by a boundary with reference to the identity of the moving water masses, the two parts into which the area of motion is thus differentiated must belong to separate and opposite eddy circulations moving in harmony with each other. It is notable in our Figure 92 that the counterpart (opposite side) of every eddy indicated in this manner is also always suggested in an appropriate spacial relationship.

DISCUSSION OF RESULTS

Two specific purposes have been aimed at in the above described attempt to analyze hydrographic profiles by a study of the distortion of the pycnomeres correlated with an analysis of identifying properties, one being concerned with the selection of a datum surface for dynamic calculations, the other having for its goal a qualitative description of the probable eddy patterns transsected by the profile, and in general the distribution of directions of motion.

With reference to the datum surface, or the basal boundary of zero motion, the results indicated in Figure 88 on the whole places this surface at very consistent density values without significant recourse to smoothing. In both profiles the motion in the direction of the main current would appear to descend to deeper isopycnic surfaces (below σ_t 27.6) at the extreme right of the segment shown in the illustration, as one gets below and beyond the main current on the surface to the open oceanic waters on the right, where the direction and velocities are probably more constant and stable throughout, and therefore capable of building up a cumulative influence to greater depths. Through the left, and probably more variable part of the system, including the region of the main current, the indicated boundary runs consistently through densities varying only between 27.26 and 27.33 in the profile off Cape Hatteras (through stations 1228–1230 inclusive, Figure 92), and between 27.32 and 27.38 in the cross-section between Cape Cod and Bermuda (through stations 1345–1349 inclusive, Figure 94). In both profiles the indicated boundary rises to lower density values at the extreme left (σ_t 27.16 at station 1231, Figure 94; σ_t 27.11 at station 1344, Figure 92). Only one single deviation from this regular pattern is indicated, namely at station 1350 (Figure 92) where the boundary as drawn by our method rises to σ_t 27.10, but this is scarcely of any great significance.

It thus seems indicated that there is a tendency for the surface of discontinuity with reference to motion to follow a constant isopycnic surface over a considerable part of the width of the current. When we again recall that the curves of $\frac{\Delta D}{\Delta \sigma_t}$ are in first approximation simply the reverse of the vertical stability curves, we further notice that the indicated basal current boundary in both profiles extends to the left along the continuation of an isopycnic surface identified with a pronounced intermediate vertical stability maximum at the right. Moving from the right-hand side towards the portions of the profiles shown in Figures 92 and 94 we find this intermediate stability maximum at density values corresponding to the basal current boundary in the left portions strongly developed through stations 1220–1227 (Figure 88) referring to the right approach to Figure 94, and through stations 1355–1351 (Figure 88) referring to the right approach to Figure 92. This interme-

diate stability maximum also remains quite pronounced through several of the nearest station intervals at the left (station 1229, Figures 88 and 94; stations 1345 and 1347, Figures 88 and 92) and it is even more or less distinctly suggested by double inflections (≀) at all the remaining stations considered in Figure 88, with the only exception of station 1344 (Figures 88 and 92) which forms a single continuous curve through the layers of densities higher than 27.00.

This relationship between the indicated current boundary and the location of an intermediate vertical stability maximum can, in the writer's opinion, be considered an independent verification of the method here suggested, since a greater vertical stability should mean a greater lateral freedom of motion through reduced vertical transfer of momentum (Parr, 1936). The break between opposite currents above and below should therefore naturally be expected to occur along the surface in which the vertical eddy viscosity would be at a minimum in comparison with the nearest adjacent zones of oppositely directed movements above and below. And when the break has once developed, the discontinuity with reference to the origin and derivation of the water masses above and below tends to become self-perpetuating.

In this instance the source of the main motion can obviously be assigned to the light water masses on the right, which carry the momentum of the Florida Current (Rossby, 1936). Where this type of water extends uniformly to great depths, we have already mentioned that we also find the indicated current boundary descending to deeper isopycnals below the intermediate vertical stability maximum. But at the left where this lighter water carrying its momentum with it extends inward towards the shore above heavier water reaching fairly high levels underneath, we find the indicated current boundary immediately rising to the isopycnal surface *along which the upper part of the uniformly moving water column at the right can be most easily displaced laterally against its lower portion.* Since this variation from right to left in the relationship between the indicated current boundary and the intermediate vertical stability maximum is exactly what one would be led to expect from the preceding considerations, it would seem to lend further support to the validity of the method of current analysis here suggested.

If we now turn to a comparison with the results obtained by Dietrich's method (Dietrich 1937a, b) of using the oxygen minimum surface as the lower boundary of the Gulf Stream system, we find an indeed surprisingly close agreement, as shown by the triple circles entered in Figure 94, to indicate the depths of oxygen minimum as determined by interpolation from reasonably drawn vertical oxygen curves. These oxygen minimum depths show no significant deviation from the indicated current boundary at any point except at the extreme right where they follow the intermediate

stability maximum surface, rather than the indicated surface of zero motion. But this deviation at the right is obviously in equally perfect agreement with our expectations as is the coincidence throughout the left portion in accordance with the interpretation offered in the preceding paragraph.

Dietrich (1937a) has further also found support for the use of the oxygen minimum surface as the lower boundary of the Gulf Stream system through a consideration of transverse movements, and here again his evidence is abundantly confirmed by the transverse circulations indicated by the relative salinity profile shown in Figure 94.

We may thus apparently claim confirmation of the actual validity of the indicated current boundary obtained by the pycnomeric method herein described from no less than four entirely independent sources. (1) From the logical premises of the method itself, which rest upon the requirement for a distortion of the pycnomeres caused in a definite manner by the actual movement itself. (2) From the fact that the indicated current boundary is located in accordance with reasonable expectations in relation to the vertical distribution of vertical stability, which is an arithmetically fortuitous result entirely independent upon the method used for determining the probable current boundary. (3) Through the perfect agreement below the main current with the results obtained by the entirely independent oxygen minimum method used by Dietrich (1937a, b), for reasons which have been fully discussed by him. (4) From the equally good agreement with the picture obtained from a study of transverse movements.

To this we may finally add a fifth qualitative confirmation from a consideration of quantitative relationships. Using the oxygen minimum surface for reference between stations 1225 and 1231 (see Figure 94, stations 1225–26 are beyond the picture to the right), Dietrich (1937, p. 512) gave a volume transport estimate for the Gulf Stream system through this profile of 31.75 million cubic meters per second. This figure agrees entirely with the estimates of the flow through the Central American Seas previously made by the present writer on the basis of four separate profiles through the Caribbean and Cayman Seas and the 24-hour averages for five anchor stations across the Straits of Florida, which all together gave as a result 30–34 million cubic meters per second (Parr, 1937, p. 48). Whether this agreement is accidental or has any dynamic significance is impossible to say, however, since it has been stipulated by Rossby (1936) that there must be an increase in volume transport downstream, which should come into effect between the Straits of Florida and the location of the profile here considered off Cape Hatteras. And this required increase is available in the results of the method here proposed.

It has already been mentioned (p. 277) that the indicated lower current boundary on the right side descends from its isopycnal on the left, which continues through the right portion as the isopycnal of minimum oxygen

and intermediate maximum vertical stability only. Between stations 1228 and 1227, and likewise between stations 1227 and 1226 (beyond Figure 94 to the right) the relationships of the $\frac{\Delta D}{\Delta \sigma_t}$ curves indicate by actually determined values fairly strong movement in the direction of the main flow down to the deepest isopycnals (densities higher than 27.6, see p. 283). Still farther to the right conditions become vague and the differences between the $\frac{\Delta D}{\Delta \sigma_t}$ curves below σ_t 27.5 are not uniquely determined by actual observations, but largely due to "reasonable" interpolations of smooth curves, and are therefore without definite significance. Beyond station 1227 to the right, we can therefore not obtain a useable reference surface by the method here suggested, but nevertheless have definite indication of "Gulf Stream flow" down to great depths at least between stations 1227 and 1226, much deeper than the oxygen minimum surface on this side. From station 1227 to the right (and also between station 1228 and 1227) we must therefore make an addition to the estimate arrived at by Dietrich, who used the oxygen minimum surface for reference also through the right portion of the series to station 1225. If we therefore follow Dietrich's method for stations 1231–1227,* but use the 2000 decibar surface as our datum level for station 1227–1225, we arrive at an indicated total volume transport of about 50 million cubic meters per second.† If we change our reference surface from the oxygen minimum to the 2000 decibar level already at station 1228, our estimate indicates about 69 million cubic meters per second (only about 65 million cubic meters per second according to Dietrich's Table 2, see footnote † below) with the gross transport in the Gulf Stream direction not very significantly higher than the net transport. Since it is indicated that the basal current boundary departs from the oxygen minimum layer between stations 1228 and 1227, the correct value for the volume transport should probably lie between these two figures, at say about 60 million cubic meters per second.

* Using the values given in Dietrich's Table 2 (Dietrich, 1937, p. 512) for the volume transport with reference to the oxygen minimum surface.

† If we use the values given in Dietrich's Table 2 (see footnote above) for the volume transport both with reference to the oxygen minimum surface and with reference to 2000 decibars at the right, the procedure gives only about 45 million cubic meters per second. The total net volume transport with reference to 2000 decibars calculated for the entire profile (stations 1231- 1225) is given by Dietrich as 68.32 million cubic meters per second. Using end stations only, with the values of anomalies of dynamic height given in Dietrich's Table 1, we obtain a little over 73 million cubic meters per second, which seems in fair agreement with Iselin's estimate of 82 million cubic meters per second gross flow in the Gulf Stream direction (Iselin, 1936).

In other words, by taking into account the fact that the indicated boundary departs at the right from the oxygen minimum layer which it follows at the left, we find that there must have been an increase in volume transport between the Straits of Florida and our profile off Cape Hatteras probably amounting to somewhere around 30 million cubic meters per second, which, since it fulfills an independent theoretical requirement not fulfilled by the oxygen minimum method alone, gives a fifth independent verification of the validity of the proposed method.

Although the probable net volume transport arrived at for the Hatteras profile is thus about twice as great as that obtained by the oxygen minimum method alone, it is still only about 75% of that calculated by Iselin by referring the motion to the 2000 decibar surface throughout (82 million cubic meters per second, Iselin, 1936, p. 75). This reduction from Iselin's estimate greatly facilitates our understanding of the current profile, particularly by virtually cancelling the need for such cumbersom explanations as that which is required to interpret the volume transport through area E as given in Iselin's diagrammatic analysis of the profile (Iselin, 1936, Figure 49, p. 78), namely, that the water masses passing through this area *in the center* of the current have been about 50% diluted in the course of only 600 miles of northward flow.

The new interpretation of the current profile off Cape Hatteras thus seems to give a completely reasonable presentation both from the quantitative as well as from four different and independent qualitative points of view.

EDDY CIRCULATION PATTERN

It has been pointed out by Iselin (1938, see also Iselin, 1936, pp. 24–25, 69) that the occurrence of a system of eddies along the left side of the current seems to be the normal rather than the unusual state of affairs in the passage of the Gulf Stream along the Atlantic coast of the United States. This conclusion is also in accordance with the theoretical expectations of Rossby (1936) and the findings of Parr (1937) and is amply confirmed by the result of our study of pycnomeric distortion.

By assigning direction of motion to eddy systems differentiated by their identifying properties as mentioned in our description of procedure (p. 282), we find two major upper eddies rotating in harmony with each other and with the steady flow at the right, and the apparently also steady flow in the same direction at the left in the profile from Cape Cod to Bermuda (Figure 92). It should be noted that velocities calculated with reference to a horizontal datum level applied to a system of this sort would indicate *only one single* eddy rotating in conflict with a steady motion at the right. In the Cape Hatteras profile (Figure 94) in which dynamic calculations with reference to a horizontal datum level would not indicate any eddy at all, our

method brings out the existence of three eddies, all rotating in harmony with each other, and with the steady current at the right and the presumably steady counter-current at the left. Iselin's findings are thus fully substantiated by the results of the pycnomeric distortion method, and the pictures offer no difficulties with reference to conflicting directions of motion.

In both Figures 92 and 94 we notice a relatively narrow, but fairly consistent belt immediately above the indicated current boundary, in which the indicated movement is generally in the opposite direction of the movement in the much deeper and quantitatively far more important upper eddies. As mentioned on page 275 the appearance of this intermediate belt may partly be due simply to the inaccuracies of the computed values in this density region where the absolute differences between the $\frac{\Delta D}{\Delta \sigma_t}$ curves are slight. But since this belt is characterized by intermediate maximum vertical stability and is closely associated with the surface along which lateral translation of the upper water masses in relation to the lower bodies of water is indicated, it is perfectly reasonable to expect a relatively thin zone of very complicated eddies. Indeed, it would seem suspicious if such eddies were not suggested by our analysis. But probably the comparatively thin eddies in this contact zone are actually much more complicated than the comparatively widely spaced stations will permit us to discover.

Below the indicated boundary, there are also laterally adjacent zones of apparently opposite movements, and slow eddying probably also occurs here, but for the deeper isopycnal surfaces our evidence becomes gradually vaguer (see p. 286) and it seems wise to refrain from drawing any definite conclusions with regard to conditions below the Gulf Stream system (see also Dietrich, 1937, p. 514).

In conclusion, it is necessary, however, to make the reservation that neither dynamic calculations as expressed in profiles of dynamic height or in dynamic topographies, nor studies of pycnomeric distortions can ever give unequivocal *a priori* evidence of the direction of motion in any eddy system, or even of the mere existence of an eddy circulation. If a body of lighter water occurs in an isolated position surrounded by heavier water on both sides (as at station 1347, Figure 92) both pycnomeric distortion and dynamic calculations with reference to any reasonable datum surface would indicate the presence of an eddy circulation, even if the isolated body was completely stationary without any rotation or any other form of motion. It is only on the assumption that such isolated bodies cannot occur in the absence of rotation without dispersing to form a uniform light surface layer that either the pycnomeric or the dynamic method can be applied. On this assumption the dynamic method alone may, as we have seen, fail to indicate the full number of rotations present, thereby suggesting conflicting movement in the contact zones, while the pycnomeric method in conjunction with

the assignment of motion according to the distribution of identifying properties will always indicate a system of eddies in harmonious rotation.

But even when we thus obtain a reasonable and harmonious picture of eddy circulation around an isolated light or heavy body of water, there will still always remain one final uncertainty in view of the fact that both the dynamic and the pycnomeric method can only indicate *rotation in one single direction* around such a body, even if this body in its historical origin has actually been split off from its parent body with a *rotation in the opposite direction* to that indicated by either method of analysis. For this difficulty there would seem to be no solution except by actual observations, and lacking such observations there is always the possibility that indicated direction of rotation may be entirely reversed in any particular instance. But such occurrences will, let us hope, in all probability not be very frequent, and lacking direct observations we must proceed on the assumption that the indicated direction of rotation will, as a general rule, correspond to the actual direction in nature.

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THE OCCURRENCE AND DISTRIBUTION OF NITRIFYING BACTERIA IN THE SEA

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Of all the phases in the cycle of nitrogen in the sea perhaps that of the oxidation of ammonia has received the most attention, and yet many questions related to this process are still unsettled. Evidences of nitrifying bacteria have been found in marine bottom materials, particularly in shallower waters, but their presence in the water and plankton of the ocean has not been established.

An attempt was made, therefore, to try to ascertain (1) whether sea water itself with no added source of nitrogen could produce nitrite and nitrate, and (2) whether there were any regions of the ocean other than the ocean floor where such bacteria were present, and nitrifying processes were taking place.

METHODS

Samples of sea water were collected from different stations and kept in the laboratory in sterile flasks for several weeks, at room temperature and in the dark. These were tested at various intervals for the presence of nitrite, nitrate, and in some cases for ammonia. Other samples of sea water were held at 80° C. for 30 minutes, and after they had absorbed sufficient oxygen again they were inoculated with different samples of fresh marine muds or with plankton. Standard sea water medium, known to be favorable for the formation of nitrites and nitrates was also inoculated with fresh marine bottom material, sea water, or plankton. This medium contained 10 g. sand, 1 g. $CaCO_3$, 40 cc. sea water, 5 mg. K_2HPO_4, and 5 mg. $(NH_4)_2SO_4$ per flask. For nitrate formation, the $(NH_4)_2SO_4$ was replaced by 5 mg. KNO_2. These cultures were also incubated at room temperature and in the dark.

Tests for nitrite were made with diethly-alpha-naphthylamine reagent. In testing for nitrate any nitrite present was first destroyed by keeping the solution, to which sulphanilic-acetic acid solution had been added in a bath of boiling water for 20 minutes, and then testing for nitrate with diphenyl amine reagent. Portions of all solutions treated in this manner were always

* Contribution No. 188.

tested again after heating to see if all nitrite had been destroyed. The nitrate test is not as sensitive as that used for nitrite. Therefore, the former cannot be detected in as small amounts by this means. The presence of ammonia was determined by using Nessler's solution in spot plates. All test tubes and pipettes used for testing for nitrite and nitrate, were washed and rinsed several times in tap and then distilled water and steamed before using.

PRESENCE OF NITRIFYING BACTERIA IN BOTTOM MATERIALS AND IN SEA WATER. MUD SAMPLES IN STERILE STANDARD MEDIA

In order to determine whether organisms contained in bottom material would oxidize ammonia to nitrite and the latter to nitrate, samples of mud were taken for the first experiment, 1 mile W. by N. of Woepecket Buoy in Buzzards Bay at a depth of 14.3 meters. The fresh mud was added to sterile standard media. This experiment was repeated using sand collected off Quissett Harbor, Buzzards Bay, at a depth of 9.1 meters. The results showed that Woepecket mud produced nitrite from ammonia in 2 days, while the nitrite thus produced was oxidized to nitrate in 35 days. Similar oxidation of the nitrite occurred in the nitrite medium. The sand, however, did not give a positive test for nitrite for 11 days. Nitrite appeared in this culture after 69 days, but no nitrate was found in the medium originally containing nitrite.

To amplify this experiment and determine more definitely the locus of nitrification a series of mud cores were obtained off Nomans Land. These cores were about 5–6 inches long. Standard media were inoculated with portions of these cores. One flask of each medium received fresh sea water taken from above the core, and two other flasks both surface mud and water directly over the core. Portions of the core at a depth of 1, 5, 10 or 12.5 centimeters were also placed in these media. (Table I.) All flasks containing ammonium sulphate medium and inoculated with mud showed the production of nitrite in 9 days or less, with the exception of the sample taken at a depth of 12.5 cm. below the surface of the mud. The nitrite increased with time so that in 30 days or less all of the cultures containing the mud inocula were showing strong tests for nitrite, except the last sample. Material taken from another core at a depth of 10 cm. gave decided nitrite tests. The media inoculated with mud and water, or with water alone were much slower in giving positive tests, and when such tests occurred they were weak. Nitrate did not appear in the nitrite medium for 2 months. After that, positive tests were obtained in the cultures inoculated with mud, but not in those receiving water, or mud and water. This corroborates previous findings, that nitrites and nitrates are produced from muds in cultures when

given a supply of ammonia or nitrite respectively (Waksman, Hotchkiss, and Carey (*24*)).

MUD SAMPLES IN STERILE SEA WATER WITH NO ENRICHMENT

Top and bottom layers of other cores obtained off Nomans Land were inoculated into sterile sea water. The water, after heating to 80° C., was placed in the refrigerator to become resaturated with oxygen; it was then

TABLE I

PRODUCTION OF NITRITE AND NITRATE FROM PROFILE OF MUD COLLECTED OFF NOMANS LAND AT DEPTH OF 31.1 METERS

	NITRITE								*NITRATE*
	Incubation. Days								*Inc. Days*
	3	*6*	*16*	*22*	*30*	*44*	*64*	*99*	*99*
a) Nitrite Medium									
1. Water above core	0	0	0	0	0	0	†	†	0
2. Surface mud and water	0	0	0	0	0	0	tr.	††	0
3. Core, 1 cm. deep	0	0	††	††††	†††††	†††††	†††††	0	†
4. Core, 5 cm. deep	0	tr.	†	††††	†††††	†††††	†††††	0	††††
5. Core, 12.5 cm. deep	0	0	0	0	0	0	0	*	
6. Control	0	0	0	0	0	0	†	†	
b) Nitrate Medium									
1. Water above core	0	0	0	0	0	0	0	0	
2. Surface mud and water	0	0	0	0	0	0	0	0	
3. Core, 1 cm. deep	0	0	0	0	0	tr.	††	†††	
4. Core, 5 cm. deep	0	0	0	0	0	0	†	†	
5. Core 12.5 cm. deep	0	0	0	0	0	0	††	†	
6. Control	0	0	0	0	0	0	0	0	

* Discarded after 64 days.

tr. = trace, † = positive test, †† = fair, ††† = good, †††† = very good, ††††† = abundant.

inoculated with the mud samples. The cultures were tested for ammonia, nitrite, and nitrate with continuously negative results up to 53 days.

Samples of mud bottom taken off Gay Head were added to 100 cc. portions of sterile sea water, as well as the water directly over the core. None of these gave any positive tests for ammonia, nitrite, or nitrate even after 53 days incubation at room temperature. This material gave positive tests for nitrite in 14 and 16 days, and for nitrate in 63 and 64 days, when inoculations were made into standard media as shown in Table I. Negative results were also obtained when "cultured" sea water, i.e., sea water which had stood for 5 days in the laboratory and had been resaturated with oxygen, was inoculated with mud and sand collected in Buzzards Bay near Woepecket Island at a depth of 9.1 meters although incubated for 59 days.

Samples of the same material gave positive tests when inoculated into standard media.

These experiments show that the presence of ammonia and nitrite-oxidizing bacteria can be demonstrated under certain experimental laboratory conditions.

To confirm this, in a second series of experiments mud from Gay Head and Buzzards Bay was added to sterile sea water and also to standard media. The depths at which these bottom samples were taken ranged from 14 to 34.7 meters. Odd numbers in the table indicate the surface layers, and even numbers the deeper layers of the core. No positive tests were obtained in the sterile water even after 52 days. In the standard medium nitrite appeared in small amounts after 5 days, and at 52 days or possibly sooner, the nitrite was being oxidized to nitrate (Table II). At the end of 90 days all the nitrite had disappeared. Complete oxidation to nitrate in the medium originally containing ammonia probably occurred before this time, but tests were not made between 52 and 90 days. Nitrate appeared in the nitrite containing medium in one case in 30 days, and in all flasks at 90 days. This again proves that enriched cultures favor production of nitrite and nitrate when inoculated with mud, whereas sterile sea water inoculated with mud does not give this reaction in the same incubation period.

To test the presence of nitrifying bacteria at greater distances from land 500 cc. portions of water obtained by the "Atlantis" from Station 2895 at depths of 10, 35, 90, 130 and 200 meters was used. Three series of flasks were prepared, one receiving fresh sea water alone; the second being enriched with 10 mg. $(NH_4)_2SO_4$, and the third containing 20 cc. of fresh sea water and 40 cc. of standard medium containing $(NH_4)_2SO_4$. The flasks were placed in the dark and tested at various intervals for ammonia, nitrite and nitrate. Nitrite and nitrate were negative up to 44 days, and tests for ammonia were positive only where this substance had been added to the water. Mud samples obtained from Station 2895 (lat. 40° 04′, long. 70° 50′) and inoculated into standard media showed, in a few cases, slight traces of nitrite after 2 weeks, but no nitrate up to 86 days. These nitrite tests, however, were only faint and not sufficient to warrant the assumption that ammonia oxidizing bacteria were present in the muds.

Similar results were obtained from bottom samples taken from greater depths, 1137–4000 meters, at latitudes of 40° 04′ to 40° 25′, and longitudes 67° 46′ to 66° 00′.

NITRIFICATION IN PLANKTON MATERIAL

In the first experiments, 10 cc. of concentrated diatom tow from Vineyard Sound was added to 250 cc. of fresh sea water containing 5 mg. K_2HPO_4 per liter. This was put into a sterile flask containing a little sterile sand.

TABLE II

PRODUCTION OF NITRITE AND NITRATE IN STANDARD MEDIA BY BOTTOM SAMPLES TAKEN OFF GAY HEAD AND IN BUZZARDS BAY

LOCATION AND DEPTH OF BOTTOM MATERIAL	NITRITE								NITRATE							
	Incubation, Days								Incubation, Days							
	5	7	14	24	30	37	52	90	90	5	7	24	30	37	52	90
1. *Gay Head 5¼ mi. W. by S. ¼ S. Depth 32.9 M. Top*	0	0	tr.	†††††	††	†††††	††††	0	†††	0	0	0	†	†	††	††
2. *Gay Head 5¼ mi. W. by S. ¼ S. Depth 32.9 M. Bottom*	0	ft. tr.	†	††	†††	†††	†††	0	†††	0	0	0	0	0	††	†††
3. *Gay Head ½ mi. S. ½ E. of 1. Depth 34.7 M. Top*	0	0	0	tr.	tr.	tr.	†††	0	tr.	0	0	0	0	0	tr.	††
4. *Gay Head ½ mi. S. ½ E. of 1. Depth 34.7 M. Bottom*	ft. tr.	ft. tr.	†	†	†††	†††††	tr.*	0	†	0	0	0	0	0	0	0
5. *Buzzards Bay 2 mi. W. N. W. Woepecket Rock. Depth 14.3 M. Top*	†	††	†††	†††††	†††††	†††††	†††††	0	††	0	0	0	0	0	0	†
6. *Buzzards Bay 2 mi. W. N. W. Woepecket Rock. Depth 14.3 M. Bottom*	tr.	tr.	††	†††	††††	†††	0**	0	†	0	0	0	0	0	0	tr.
7. *Buzzards Bay 1 mi. N. W. by W. Woepecket Rock. Depth 14.6 M. Top*	†	†	††	††††	††††	†††	**v.f.tr.	0	††	0	0	0	0	0	0	tr.
8. *Buzzards Bay 1 mi. N. W. by W. Woepecket Rock. Depth 14.6 M. Bottom*	tr.	tr.	†	††	†††††	††††	0*	0	†	0	0	0	0	0	0	0
9. *Buzzards Bay ⅛ mi. N. Woepecket Rock. Depth 14.0 M. Top*	††	††	†††	††††	†††††	†††††	0**	0	††	0	0	0	0	0	0	†
11. *Buzzards Bay 1 mi. N. Gong 6. Depth 14.6 M. Top*	tr.	†	††	†††	†††††	†††††	†††††	0	††	0	0	0	0	0	†	tr.
14. *Buzzards Bay Buoy 42. Depth 33.8 M. Bottom*	0	0	tr.	†	††	††	†††	0	††	0	0	0	0	0	0	††
15. *Control*	0	0	0	0	0	0	0	0	†	0	0	0	0	0	0	0

* = Nitrate †; ** = Nitrate †††; Ft. tr. = faint trace, tr. = trace, † = positive test, †† = fair, ††† = good, †††† = very good, ††††† = abundant.

The control flask contained no plankton. These flasks were incubated in the dark. Nitrite appeared after 20 days, and became very strongly positive, while ammonia decreased and finally gave no test. No nitrate was produced up to 64 days. The controls all remained negative.

Another series of flasks were set up using unfiltered fresh sea water from Woods Hole, a little sand, and 5 mg. K_2HPO_4 per liter. Two hundred and fifty cc. portions of this water were put into each flask. Two flasks were inoculated with 10 cc. of concentrated diatom tow collected from Woods Hole or Vineyard Sound. The plankton consisted largely of *Rhizoselenia* and some *Guinardia*. In about 4 days ammonia was found in the flask inoculated with the sample of tow from Woods Hole. The ammonia in the flask increased for a time, and in 2 weeks nitrite appeared and became very strong after 29 days, when the ammonia test became negative. The flask inoculated with tow from Vineyard Sound, gave the same results, but produced ammonia more slowly. Nitrate was not present in either flask up to 29 days.

A third series of flasks was set up using, in one case, standard medium for the production of nitrite, and in the other case the same medium without ammonium sulphate. These were inoculated with 0.5 cc. of concentrated diatom tow. Ammonia was soon found in the flask containing tow and no ammonium sulphate. Nitrite appeared in both flasks, more rapidly, however, in the flask containing only plankton as a nitrogen source, but after 13 days they each gave equally positive tests. With no added ammonia the nitrite test did not finally become as strong, and after 27 days it disappeared; this was probably due to its utilization by other organisms, as no tests for nitrate were obtained up to 43 days. The flask with 5 mg. $(NH_4)_2SO_4$ showed decidedly positive tests for nitrite (3+) even up to 43 days. No nitrate was produced in this flask either. The controls were negative throughout.

Following these experiments plankton tow was collected from three different stations and added to sterile sea water. Tests were made at different intervals for nitrite, nitrate and ammonia. The first sample was taken from Vineyard Sound between Woods Hole and Tarpaulin Cove, and consisted almost entirely of copepods, a very few diatoms being present. Two tenths, 0.5, 1.0, 2.0, and 5.0 cc. of tow were added to 100 cc. portions of water. When tested 2 days after inoculation by the spot plate test ammonia was present increasing in concentration with the increase in the amount of plankton added. Nitrite first appeared in the flask containing the smallest amount of tow after three weeks. After 27 days the flasks receiving 0.5 and 1.0 cc. portions of the tow showed nitrite, and after 44 days a trace was present in the 2 cc. amount (Table III). Up to 94 days no nitrite had appeared in the flask containing 5 cc. of tow. No nitrate appeared in any culture up to 94 days. As the nitrite increased in the cultures

TABLE III

PRODUCTION OF AMMONIA AND NITRITE IN STERILE SEA WATER ON ADDITION OF COPEPOD TOW FROM VINEYARD SOUND

Flask No.	Plankton cc.	AMMONIA — Incubation, Days											NITRITE — Incubation, Days										NITRATE — Inc. Days		
		2	11	15	20	23	27	32	44	52	74	94	2	11	15	20	23	27	32	44	52	74	94	2	74	94
1	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0.2	†	††	†	†	†	†	0	0	0	0	0	0	v.f. tr.	tr	†	†††	††††	†††††	†††††	††††	†††††	††††	0	0	0
3	0.5	††	†††	††	††	††	††	††	†	†	†	†	0	0	0	0	0	†	††	††††	††††	††††	††††	0	0	0
4	1.0	†††	†††	††	††	††	††	††	††	††	†	††	0	0	0	0	0	†	††	††††	††††	††††	††††	0	0	0
5	2.0	††††	††††	††††	††††	††††	††††	†††	†††	†††	††	††	0	0	0	0	0	†	†††	†††††	†††††	†††††	†††††	0	0	0
6	5.0	†††††	†††††	†††††	†††††	†††††	†††††	†††††	†††††	††††	††††	†††	0	0	0	0	0	0	0	0	tr.	tr.	†	††	0	0	0

Medium contained 100 cc. sterile sea water with varying amounts of copepod tow. v.f.tr. = very faint trace, tr. = trace, † = positive test, †† = fair, ††† = good, †††† = very good, ††††† = abundant.

the ammonia decreased. The nitrite test became very strong in the cultures as they became older. Apparently nitrite producing organisms must have been present in the tow, and the decomposition of the copepods furnished the ammonia necessary for the oxidation to nitrite. If this ammonia is produced in too large quantities it retards the production of nitrite. Five drops of this tow were added to flasks of standard media for the production of nitrite and nitrate. Decided tests for nitrite were obtained in 27 days, but no nitrate was produced up to 95 days.

Tow was collected by the "Asterias" (Table IV) by towing 5 minutes with a No. 20 mesh net 10 miles northeast of Chatham. This tow consisted largely of Dinoflagellates, *Ceratium tripos*, and *Ceratium fuscus* as well as a

TABLE IV

PRODUCTION OF NITRITE IN STANDARD MEDIUM WITHOUT AMMONIUM SULPHATE BY DINOFLAGELLATE TOW

Flask No.	*Plankton*	*AMMONIA*							*NITRITE*							*NITRATE*			
		Incubation, Days							*Incubation, Days*							*Inc., Days*			
		4	*6*	*9*	*16*	*28*	*57*	*79*	*4*	*6*	*9*	*16*	*28*	*57*	*79*	*4*	*28*	*57*	*79*
1	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	2 drops	†	†	†	0	0	0	0	0	tr.	†	†	†	†	††	0	0	0	0
3	5 drops	†	†	†	†	tr.	0	0	0	0	0	0	0	†	††	0	0	0	0
4	10 drops	††	††	††	††	††	tr.	0	0	0	0	0	0	†	††	0	0	0	0
5	1 cc.	††	††	††	††	††	†	tr.	0	0	0	0	0	0	††	0	0	0	0

Tr. = trace, † = positive test, †† = fair, ††† = good, †††† = very good, ††††† = abundant.

few copepods. The tow was concentrated by filtering, and added to standard medium not containing any ammonium sulphate in the following amounts: 2, 5, 10 drops and 1.0 cc. Ammonia was present when the tow was first tested, namely 4 days after it was obtained, and nitrite appeared in the flask containing 2 drops of tow after 6 days, while after 8 weeks it was present in all flasks in small amounts, except in the flask containing 1.0 cc. of tow. At this time the ammonia had largely disappeared. The nitrite continued to increase up to 79 days. However, no nitrate was formed.

Tow collected by the "Atlantis" at 100 meters depth, Station 2895 lat. 40° 04′, long. 70° 50′, off the Continental Shelf contained large amounts of copepods and other animal forms, very few diatoms and a considerable bacterial population, by the time it was received in the laboratory. This concentrated tow was also added to 100 cc. portions of sterile sea water in the following amounts: 2, 5, and 10 drops, and 1.0 cc. Ammonia was present from the beginning of the experiment, decreasing with time, but no nitrite appeared up to 70 days. Neither was there any nitrate produced up to 100

days, when this tow was inoculated into standard media without ammonium sulphate, although plenty of ammonia was produced from the decomposition of the copepods. The ammonia gradually disappeared without the production of nitrate, but very small amounts of nitrite were formed after 100 days.

The lack of production of nitrite in the copepod tow from Station 2895 is difficult to account for. This tow was collected off the Continental Shelf and at a depth of 100 meters. It may be that the reason that nitrification takes place in the tow from Vineyard Sound and off Chatham is that there is more mixing of the water, so that nitrifying organisms from the bottom get into the plankton layer and the presence of ammonia in sufficient amounts from the decomposition of the plankton permits of nitrification. Since it is difficult to demonstrate the presence of these organisms in the water mass as a whole, it is possible that they may come to inhabit this particular layer because of some peculiar fitness of the environment there. There must be some ammonia produced here due to the decomposition of the dead cells in the plankton, and this may be the reason for their presence. It would seem that they must multiply in this layer, or at least be more plentiful there than in the rest of the water. If they are merely the result of the mixing of the water, or of washing in from land, one would think that on enriching and incubating other parts of the water mass, it would be possible to demonstrate their presence. It is, of course, also possible that still longer incubation periods are necessary to show the presence of these organisms.

DISCUSSION

The presence of nitrifying bacteria in sea water, their origin, the particular part of the sea they inhabit, and the role they play, has long been a matter of uncertainty. In 1899, Vernon (*22*), while studying the marine organisms of the Gulf of Naples, suggested that nitrification in the sea might be due to bacteria. Brandt (*2, 3, 4, 5, 6* and *7*) believed that he had proved the presence of these organisms when he obtained nitrification in marine muds in the presence of ammonium salts. Neither Gran (*9, 10*) nor Nathansohn (*17*) were able to obtain similar results in either the Gulf of Naples or Norwegian waters, but Gran obtained positive results from bottom material near shore. These two investigators concluded that the nitrifying organisms were brought in from land. Thomsen (*20, 21*) suggested that the absence of organisms in the sea water was due to the fact that sea water contained only traces of ammonia, while bottom material, due to the decomposition of plant and animal remains, contained greater concentrations of this compound. Brandt, on the other hand, argued that if nitrate came into the sea from the atmosphere or from land by way of the rivers and streams, etc., one would expect to find the highest nitrate content in the upper layer

of the water rather than in the deeper. This is contrary to actual findings, and therefore, he concluded that nitrates must be made in the sea bottom. Thomsen was able to demonstrate the presence of nitrifying bacteria in the sea bottom, but failed to find them in the sea water, plankton, or on fixed algae. He obtained nitrification in Kiel Fjord and in the Bay of Naples near land. Nitrates were only produced from samples taken near land. Issatchenko (*14*, *15*) found nitrifying bacteria in bottom material from the Arctic Ocean and Black Sea, but could not find them in surface waters. Neither could Berkeley (*1*) nor Lipman (*16*) find nitrifying forms in sea water, even after several months incubation. Lipman considered calcareous sand favorable for this oxidation process. Issatchenko also believed that sandy bottoms were more favorable for it.

Harvey (*11*, *12*, *13*) found that the quantity of nitrate in the English Channel was closely related to the production of phytoplankton, so that when the phytoplankton was at its maximum, nitrate was very low. In certain cases he obtained an increase of nitrate on storing. He concluded that sea water near the bottom contained nitrate producing bacteria. Rakestraw (*18*, *19*) found a maximum of nitrate in August in the Gulf of Maine at about 40 meters depth. Nitrite was found to increase immediately after the summer growth period. Variations of nitrite in amount and distribution apparently depend on the season and the mixing of the water.

The findings in the present paper substantiate the previous results obtained by Carey and Waksman, and Waksman *et al* (*8*, *22*, *24*), where nitrite and nitrate were found to be produced from bottom samples, in the presence of an adequate nitrogen supply, and not in water under the same conditions. Nitrate was also produced from the same material but more slowly than nitrite. No attempt was made at that time to determine whether the plankton layer might contain bacteria responsible for nitrite and nitrate production.

Nitrite is an intermediate stage in the oxidation of ammonia to nitrate. Diatoms are known (ZoBell (*25*)) to reduce nitrate to nitrite, whereas the nitrifying bacteria build up nitrite and nitrate from ammonia. Various marine bacteria are able to reduce nitrate to nitrite, or ammonia or even to atmospheric nitrogen. Therefore, the mere finding of nitrite in sea water does not tell the manner of its formation.

Ammonia is the product of decomposition of the dead plankton cells. It is produced in sufficient amounts to provide a menstruum for considerable oxidation by the nitrite organisms. The amount of ammonia present in the culture determines the rapidity with which it is oxidized. With only a small amount of plankton added to each flask, only a small amount of ammonia was produced and the oxidation occurred quickly, but with larger amounts of plankton the production of nitrite was considerably retarded.

It is difficult to explain why nitrite-forming bacteria are found in some plankton masses and not in others. The plankton which gave positive tests for nitrite was collected near shore in relatively shallow places. Collection from deeper water, off the Continental Shelf, given only slight nitrite tests. The occurrence of nitrifying organism in the plankton layer may be due to vertical mixing in the shallow regions, whereby such organisms might be brought into the upper layers of the water; or it is possible that they may multiply in the layer being very sparsely distributed throughout the sea. It is, of course, possible that such organisms may be washed in from land, and thus get into the plankton near shore. The fact that they do occur in certain plankton masses and not in others, together with the fact that this may be another locus for these organisms is of interest and warrants further study.

In the experiments reported in this paper, it was found that nitrite and nitrate were produced from samples of marine bottom in considerable amounts, when these samples were taken near land. Less nitrite was produced by similar samples taken from deep water. Nitrate was not formed by the latter samples, and its production in all cases was slower than that of nitrite.

Sea water alone apparently cannot produce nitrite or nitrate, at least in the time allowed in these experiments (52 days). It is possible, however, that nitrifying organisms are present throughout the mass of sea water, but in much fewer numbers than in the sea bottom or plankton. If this is the case, it would require a much longer period of time in the laboratory, using sea water only, for such organisms to build up sufficient amounts of nitrite and nitrate from the small amounts of ammonia present in sea water, but under natural conditions and over long periods this would add a considerable amount of nitrate to the sea

The addition of diatom or copepod plankton to sterile sea water caused the production of nitrite, when such samples were collected near land. Plankton collected farther out, off the Continental Shelf, was apparently less active, perhaps due to the lack of mixing of the water. No nitrate was produced in plankton under any conditions.

These observations lead one to conclude that nitrifying organisms are found in marine bottoms, and that by mixing of the water they may be brought into the plankton layer, where by virtue of the death and decomposition of the plankton, ammonia is liberated which these organisms may then oxidize. The lack of rapid nitrite formation in plankton off the Continental Shelf would lead to the conclusion that fewer nitrifying organisms are present there, due perhaps to lack of vertical mixing of the water. If the organisms in such a plankton are exceedingly few, a longer time of incubation would be required to determine their presence.

SUMMARY

1. Bottom material produced nitrite when inoculated into standard media. This was later oxidized to nitrate. The time required to accomplish these oxidations varied with the sample. Nitrate production was in all cases slower than that of nitrite.

2. Bottom material produced nitrate from nitrite in standard sea water media.

3. Water directly above the mud produced nitrite, but much more slowly then the mud itself, only traces being found after two months. No nitrate was produced from water over cores after three months incubation.

4. Bottom samples which produced nitrite and nitrate in standard media failed to do so in sterile sea water containing no added ammonia or nitrite salts, even after an incubation of 52 days.

5. Water samples collected away from land at depths of 10, 35, 90, 130, and 200 meters failed to produce nitrite after 44 days even when inoculated into standard media, or when enriched with ammonium sulphate.

6. Samples of mud from great depths produced nitrite very slowly and in exceedingly small amounts, and no nitrate in the time allotted to this experiment (i.e., 86 days).

7. Concentrated diatom tow collected from Woods Hole and Vineyard Sound and inoculated into sea water containing phosphate and sterile sand produced ammonia which was oxidized to nitrite, but no nitrate in 29 days.

8. Concentrated diatom tow added to sterile standard media with and without ammonium salts also caused the production of nitrite but no nitrate in 43 days.

9. Concentrated copepod tow from Vineyard Sound inoculated into sterile sea water in varying amounts gave decided production of nitrite. The greater the amount of tow added to the flasks, the longer was the time of appearance of the nitrite. The latter was produced at the expense of the ammonia, which decreased in amount as the nitrite increased.

10. Nitrite was also produced in standard media by copepod tow from Vineyard Sound. No nitrate was formed after three months incubation.

11. Dinoflagellate plankton, when inoculated into sterile standard media without ammonium sulphate, produced ammonia and nitrite, but neither substance was produced in as large amounts as in the copepod tow, probably due to the higher nitrogen content of the latter. No nitrate was produced from this plankton.

12. Copepod tow collected off the Continental Shelf at a depth of 100 meters and inoculated in varying amounts into sterile sea water and standard media without ammonium sulphate produced considerable ammonia and only very slight traces of nitrite after a long period of incubation.

The writer is indebted to Dr. Selman A. Waksman for his kindly interest and advice.

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ON THE HYDROGRAPHIC MECHANISM OF THE SO-CALLED BROWN ZONES ASSOCIATED WITH TIDAL GLACIERS

By

C. H. HARTLEY AND M. J. DUNBAR

The presence of zones of muddy, ice-free water at the sea faces of tidal glaciers has been recorded from Alaska (Gilbert, 1910), and from Spitzbergen (Stott, 1936). In view of their great significance in local ecology an understanding of the causes of these so-called "Brown Zones" seemed desirable and the problem of their establishment was first attacked hydrographically by the Oxford University Expedition to Spitzbergen, in 1933.

The present work was carried out in West Greenland, on two fjord-glaciers, Eqip Sermia and Kangilerngatâ Sermia, in Atâ Sound, the north-east corner of Disko Bay (Figure 96), in the summer of 1936. The authors take this opportunity of expressing their gratitude for the permission and help given to them by the Danish Administration of Greenland, without which the work would of course have been impossible.

Methods. It was not always possible or advisable to manoeuvre the small 20-foot motor boat, used for the work, through the close brash ice that surrounded the brown zone, and it was therefore necessary to choose days on which the brash ice was at its thinnest. The graphs published here were made from data taken on three separate days, one at Kangilerngatâ and two at Eqip. The closing water-bottle used was lent by the Admiralty, in London. The salinities were estimated by titration against silver nitrate, using Knudsen burettes.

The Brown Zones. Each of the glaciers has one brown zone at its face; the topographical relations of brown zone, brash ice, glacier, and the fjord water outside, are shown in the sketch-map (Figure 97). Inside the zone itself there is a surface current away from the ice, with a speed of about half a knot. The water is very muddy, of a coffee colour, and on reaching the surrounding brash ice, disappears under it, so that the boundary of the zone is clearly marked. Planktonic Crustacea, whose normal upper limit of vertical distribution is at a depth of between 15 and 25 metres, are found at the surface in the brown zone, in considerable numbers, a condition that makes the zone an important feeding area of the Kittiwake and other sea-birds. From the fact that the Kittiwake are always crowded together immediately under the face of the glacier, it is inferred that the water rises up the face of the ice itself. Unfortunately it is not possible to work a hydrographic station nearer than 100 yards from the glacier. The distance, at Eqip, from the ice cliff to the end of the brown zone is about 250 yards.

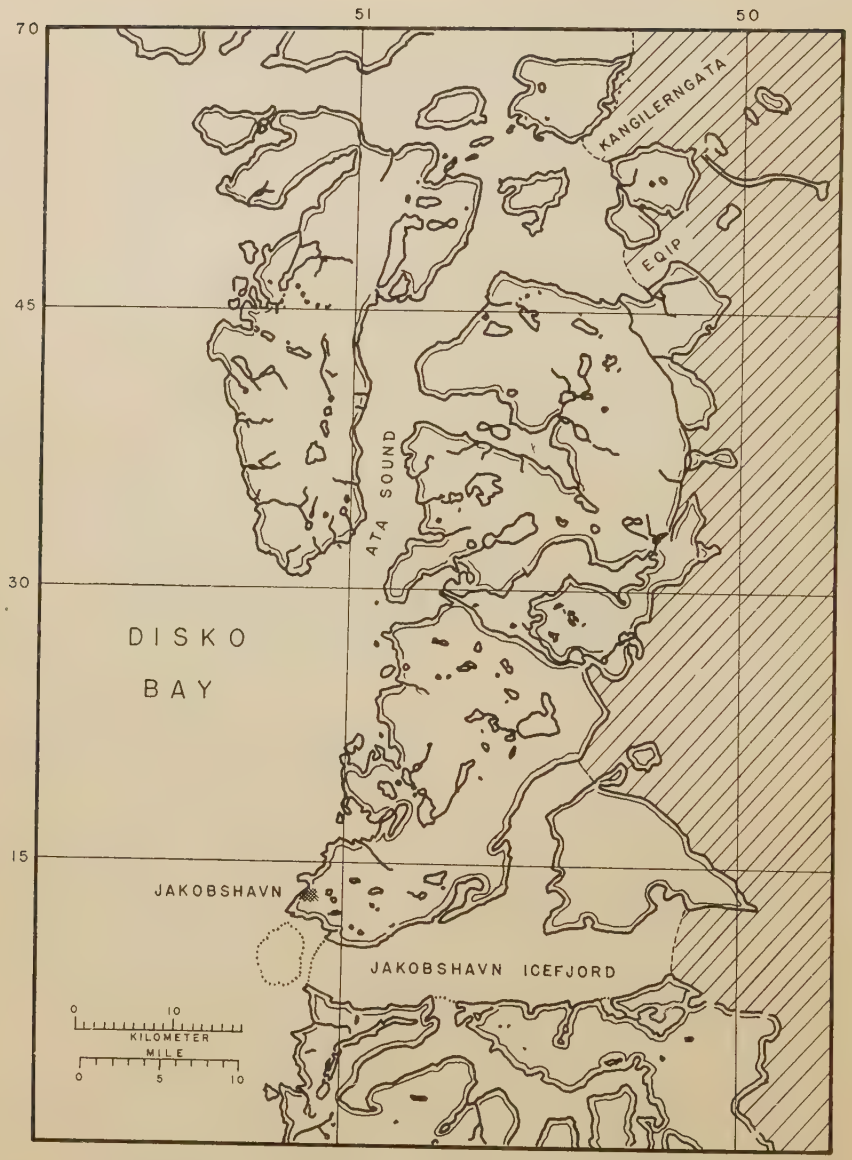

Figure 96. Chart showing location of tidal glaciers. Ice represented by hatching.

From observations made at the Nordenskiold Glacier in Spitzbergen, Stott, (1936) was led to the belief that the brown zone there was due to a drainage stream from the glacier, at or near the surface. The temperature, salinity, and density graphs, and the temperature-salinity correlation curves published here (Figures 98, 99) give no evidence whatsoever of such a stream entering the system, although the possibility is not excluded. Unfortunately the low and small range of temperatures met with in Greenland makes the density-salinity correlation curves useless, as far as the demon-

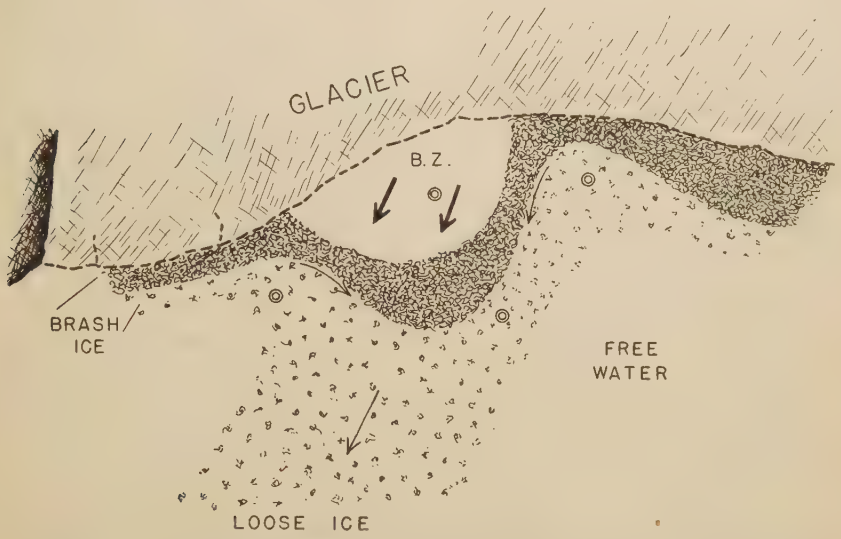

Figure 97. Sketch map of the Eqip Brown Zone, August 22, 1933. Points of observation indicated by double circles. Directions of surface movement by arrows. BZ = Brown Zone. Note absence of significant freshening of Brown Zone at this time shown in Figure 98.

stration of freshening along isopycnic surfaces is concerned. The σ_t-S curves are all virtually coincident, rectilinear and of the same slope. The negligible difference in salinity between the surface and the deeper layers within the area of the Brown Zones at Kangerlingata on August 3, and at Eqip on August 22 (see tables and Figure 98) shows an absence of significant freshening in the ascending motion which must be assumed to be associated with these zones, while the high surface salinities in themselves exclude the possibility of a fresh drainage stream entering at the top. It is therefore evident that the existence of the brown-zone can not be dynamically dependent upon the presence of a glacier stream entering at any level within the area of the zone itself. That a freshening *may* occur also within the Brown Zone as shown by the observations at EQIP on August 9, thus seems merely an incidental

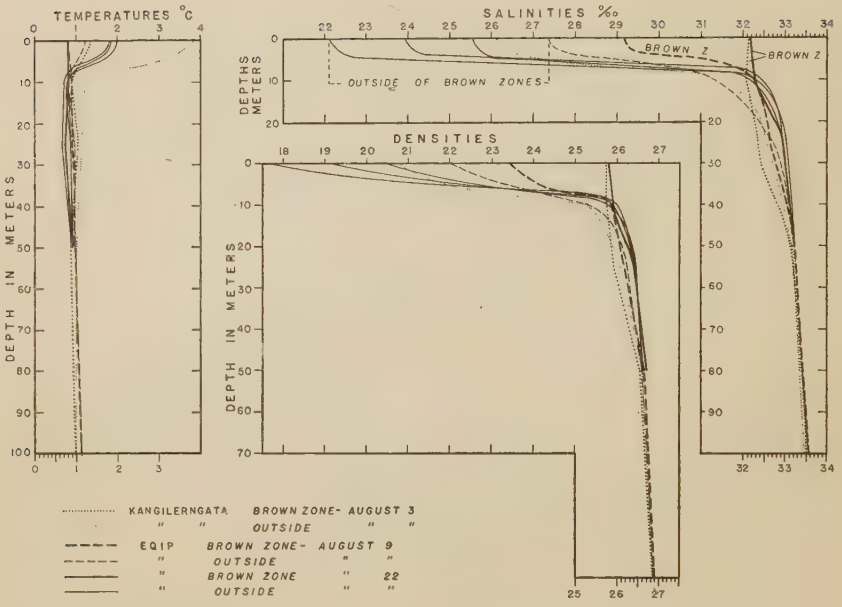

Figure 98. Temperature, Salinity and Density curves for stations inside (heavier lines) and outside (light lines) of Brown Zones.

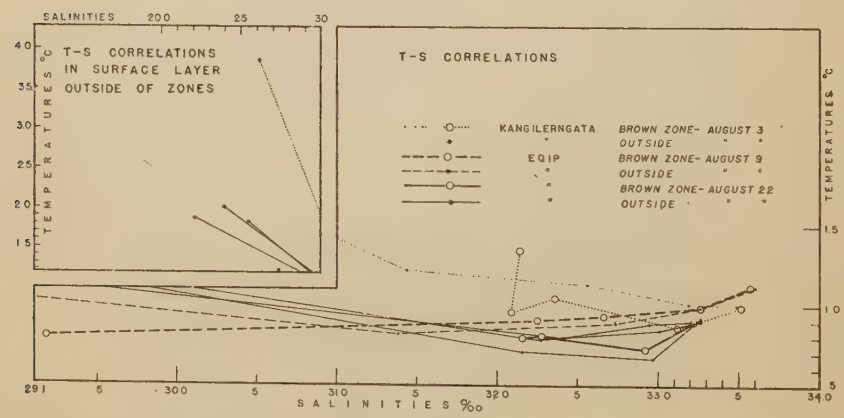

Figure 99. Temperature–Salinity correlation curves for stations inside and outside of Brown Zones.

and additional feature in this particular case but not a fundamental requirement for the existence of the zone as such. The primary force which causes the establishment of the equilibrium must therefore be sought outside of the Brown-Zone itself. Since the surface salinities observed immediately outside of the surrounding brash ice are in all instances (see tables and Figure 98) much lower than the surface salinities within the Brown Zones, it is obvious that the actual freshening is mainly or entirely introduced in or *via* the brash ice belt. The primary cause for the maintenance of the Brown Zone phenomenon is therefore to be found in the movement away from the

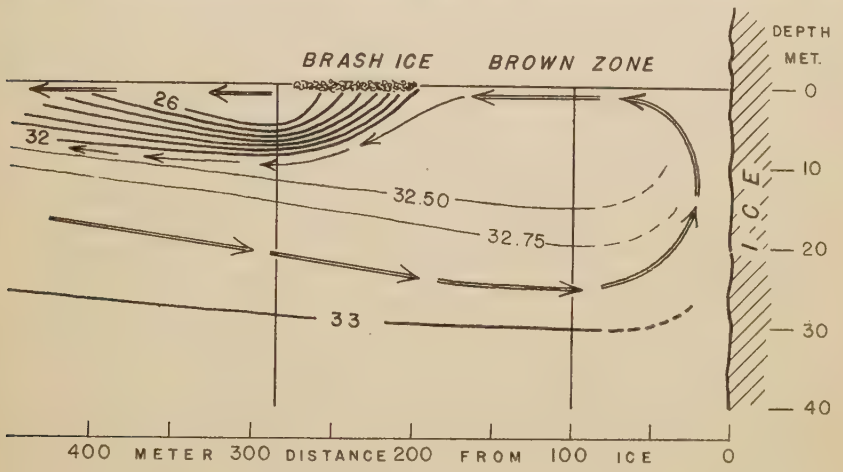

Figure 100. Indicated salinity profile (heavy isohaline for every 1 °/₀₀, from 26 °/₀₀ to 33 °/₀₀, light isohalines for 32.5 and 32.75 °/₀₀) and vertical circulation at Eqip Brown Zone, August 22, 1933.

glacier of the fresh layer occurring in the brash ice belt, where it is probably mainly derived from the melting of the brash ice itself. The system thus established is diagrammatically illustrated in Figure 100.

The upwelling water which replaces the melt-water, in the hypothetical beginnings of the brown zone in the Spring, might conceivably be in the form of a sheet, rising all along the face of the glacier. It is probable, on physical grounds alone, that such a sheet would soon take the line of least frictional resistance and form itself into a column. The presence of a drainage stream, however, be it never so small, would facilitate this process, by serving as a centre of concentration. Moreover, there are three other considerations which favour the belief that such a stream is present:—

1. The normality of a drainage stream; most glaciers possess one at least. (Gilbert, 1910; Von Engel in, 1911; Antevs, 1922.)

TABLE I

EQIP GLACIER

August 9, 1936

Depth Meters	Brown Zone			Outside A		
	°C	°/₀₀S.	σ_t	°C	°/₀₀S.	σ_t
0	0.80	29.18	23.41	1.22	27.38	21.94
10	0.91	32.25	25.87	0.82	31.38	25.17
25	0.93	32.66	26.19	0.99	32.74	26,25
50	0.99	33.24	26.65	0.99	33.26	26.67
100	1.12	33.57	26.90	1.12	33.60	26.93

TABLE II

EQIP GLACIER

August 22, 1936

Depth Meters	Brown Zone			Outside: A		
	°C	°/₀₀S.	σ_t	°C	°/₀₀S.	σ_t
0	0.80	32.16	25.80	1.86	22.11	17.71
10	0.81	32.28	25.895	0.80	32.41	26.00
25	0.73	32.92	26.41	0.89	32.95	26.42
50	0.90	33.22	26.63	0.90	33.21	26.63
Depth Meters	Outside: B			Outside: C		
	°C	°/₀₀S.	σ_t	°C	°/₀₀S.	σ_t
0	1.81	25.55	20.49	2.00	23.95	19.17
10	0.71	32.16	25.80	0.79	32.27	25.885
25	0.67	32.97	26.45	0.85	33.01	26.47
50	0.92	33.26	26.67	0.91	33.26	26.67

TABLE III

KANGILERNGATA GLACIER

August 3, 1936

Depth Meters	Brown Zone			Outside A		
	°C	°/₀₀S.	σ_t	°C	°/₀₀S.	σ_t
0	1.35	32.14	25.74	3.85	26.13	20.79
10	0.96	32.09	25.735	1.22	31.44	25.19
25	1.05	32.36	25.94	1.13	32.56	26.09
50	0.87	33.12	26.56	1.01	33.19	26.61
100	0.99	33.51	26.87	1.00	33.49	26.85

2. The presence of the mud. It has been suggested that the vibration caused by a moving glacier might keep the bottom sufficiently stirred up to supply the mud. The muddy water at Eqip, however, is found at the surface and at 10 metres depth only, in the brown zone, and at 10 metres depth outside the zone, in the direction of the brown zone current; it is not found at 25 metres or lower either in the zone or out of it. It seems, therefore, that the turbidity is not due to a general stirring up of the bottom by water movement or any other phenomenon, but to the presence of a localised source, such as a drainage stream emerging from the glacier. The supply of mud need not be very great, since (Figure 100) a large amount of it may be kept in circulation by means of its sinking from the outgoing flow at the surface to the incoming and ascending flow underneath.

3. The position of the brown zone. The Eqip brown zone is well toward the north-west end of the glacier face. If there were no drainage stream to localise the zone, one would expect it to be found more nearly in the middle.

The authors are confident that further work will solve the problem of the implication of a drainage stream in the system. They wish to express their gratitude here to Dr. A. E. Parr for valuable suggestions and assistance in the presentation of the data.

SUMMARY

Investigation of the so-called Brown Zones (zones of upwelling water at the sea faces of tidal glaciers) at two glaciers in West Greenland has shown that the upwelling cannot be due primarily to drainage streams of fresh water from the ice, as previously suggested, but that there is reason to suppose that drainage streams, although insignificant in terms of volume, may play some part in the system. It is suggested that the maintenance energy requirements of the phenomenon are derived from the movement of meltwater away from the glacier, and not from the effects of a fresh water drainage stream emerging from the ice.

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BACTERIA AS FOOD FOR CERTAIN MARINE INVERTEBRATES

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Because of their ability to convert dissolved organic matter into cell substances and to synthesize protoplasm below the depths to which radiant energy penetrates sea water, bacteria may play an important role in the nutrition of marine animals. It has been well established experimentally that bacteria serve as an important source of food supply for protozoa (Luck et al, 1931) but it is not definitely known to what extent they may play a role in the nutrition of higher forms of animal life. The solution of this problem requires information on several different points: (1) What animals in the sea may ingest microorganisms? (2) Are the ingested microorganisms assimilated and to what extent may they nourish these animals? (3) Are bacteria sufficiently abundant to constitute a significant part of the food supply of the animals? (4) Are all marine bacteria equally nutritious and may some species indigenous to the marine environment be toxic or poisonous to the animals which ingest the bacteria? Some of these questions are answered by the field observations and laboratory experiments discussed below.

EXPERIMENTS WITH MUSSELS

In the first series of experiments the sea mussel, *Mytilus californianus*, was selected for study as a representative of the lamellibranchs which are believed to derive their nourishment primarily from the finely divided detritus which they swallow (Field, 1922). *M. californianus* is quite abundant in this vicinity, specimens are of convenient size, they grow fairly rapidly, they can be weighed and measured with a fair degree of accuracy and they can be maintained in the laboratory with relative ease.

Healthy mussels measuring 6–9 cm. in length and weighing 20–50 gm. were placed after scraping their shells clean in battery jars containing sea water aged in the dark. The organic matter content of the aged sea water was negligible (less than 2 mg. per liter) after it had been filtered through paper to remove particulate matter and held in the laboratory in the dark for several weeks. After two weeks' starvation the mussels were placed singly in 600 cc. of aged sea water and offered a bacterial meal by adding a suspension of washed bacteria.

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For the initial tests two species of bacteria were selected which have distinctive morphological and cultural characteristics by which they can be readily recognized, namely *Rhodococcus agilis* Ali-Cohen and *Bacillus marinus*, n. sp. The former is a motile Gram-positive coccus 0.8 to 1.0 μ in diameter, occurring singly, in pairs and occasionally in fours. On nutrient sea water agar it forms small, circular, rose-red, glistening colonies. *B. marinus* is an aerobic Gram-positive rod 0.8 to 1.8 μ by 7.0 to 9.2 μ, forming central spores which are 1.2 to 1.8 μ by 2.4 to 3.6 μ. The cells occur singly and in chains. It is motile by means of peritrichous flagella. On sea water agar the grayish-white, finely granular, glistening colonies are 5 to 8 mm. in diameter. Large quantities of the bacteria were grown on nutrient sea water agar in Blake bottles incubated at 25° C. Two-day old cultures were harvested and washed by centrifuging in two changes of isotonic salt solution to remove dissolved and adhering nutrients.

Sufficient washed bacteria were given to the mussels to render the water perceptibly turbid (between 500 and 5000 million per cc.). Larger concentrations of bacteria were not well tolerated by the mussels unless the water was oxygenated by bubbling air through it. The mussels removed most of the bacteria from suspension within two hours as indicated by the clearing of the water, whereas in the control jars lacking mussels the water remained turbid for several hours.* Plate counts revealed that the bacterial population was reduced to only a few hundred thousand bacteria per cc. within six hours. However, several thousand or more bacteria per cc. always remained suspended. This is partly attributed to the imperfections in the filtering mechanism of the mussel but mostly to the fact that bacteria multiply at the expense of the metabolic waste products of the mussel. After a day or two virtually all of the two test organisms disappeared or their presence was obliterated by the mixed microflora which developed. It is believed that Moon and Hamilton (1934) underestimate the ability of fresh-water mussels to remove bacteria from water because they overlooked the fact that the excretory products of well-nourished mussels provides for the multiplication of bacteria.

Part of the bacteria from the suspension were merely clumped by the mussels and rejected as pseudo-feces (Dodgson, 1928) but a good many of them were actually ingested. This was proved by the microscopic and cultural demonstration of the bacteria in the digestive tracts of mussels which were dissected within an hour or two after receiving a bacterial meal. The stomachs and especially the head of the style of the mussels which were fed with the red coccus were tinted pink by the ingested bacteria. The intestinal tracts of the control mussels were virtually sterile after two weeks' starvation.

* It has been shown by Fox, Sverdrup and Cunningham (1937) that mussels of this size filter between one and two liters of water per hour.

Most of the bacteria disappeared from the intestinal tracts of the mussels within six hours after being fed. Direct microscopic examinations showed that there were very few more bacteria per unit volume of extruded feces than in the surrounding water, thereby indicating that the bacteria had been digested by the mussels. In fact, bacteria in different stages of lysis were observed in the stomach contents. Incidentally there was a much larger proportion of spores of *B. marinus* in the pseudo-feces of mussels fed on this species than in the original suspension. This may be interpreted as showing a selective exclusion of spores unless spore formation is stimulated in the pseudo-feces by the conditions of the experiment. The spores which were ingested were digested only slowly or not at all because they disappeared very slowly from the intestinal tract and they were many times more concentrated in the feces of the animals than in the surrounding water.

Digestive enzymes which lyse bacteria were recovered from the viscera of mussels. The digestive diverticula, stomachs, styles and intestines of several large well-nourished mussels were dissected free of other tissue and triturated with sand. The resulting extract was filtered through four thicknesses of cheesecloth and preserved in the refrigerator with toluol. A drop of the extract was added to a drop of bacterial suspension in small vials. After two to six hours' incubation at 25° C. stained smears of the mixture were examined microscopically. The cells of all except three of the 31 different species of marine bacteria tested were dissolved by the extract. When the extract was inactivated by heating to 75° C. for ten minutes the bacteria were merely agglutinated or unaffected.

The bacteria which were not attacked by the digestive enzymes of the mussel included two undescribed species of acid-fast *Mycobacteria* and an *Actinomyces*-like rod. The vegetative cells of *Bacillus marinus* were rapidly lysed and its spores seemed to be slowly attacked by the enzyme extract. Incidentally most of the other bacteria were Gram-negative *Bacteriaceae*. No difference was noted between the effects of Gram-positive and Gram-negative bacteria on the mussels. Oehler (1916, 1920) reports that Gram-positive species are generally digested less readily than Gram-negative ones and that certain acid-fast bacteria and spores are not digested at all by flagellates or ciliates. After investigating the digestibility of numerous bacteria by soil protozoa Severtzova (1928) concluded that small non-sporulating rods and cocci are the best sources of food while the large spore-forming rods are not so useful, although the vegetative cells of the latter are readily ingested and digested.

Having found that mussels ingest bacteria and that they possess enzymes capable of digesting bacteria, experiments were next designed to ascertain if the mussels might derive any nutriments from the bacteria. For this purpose specimens 2–4 cm. long and weighing 2–5 gm. each were collected. The small mussels were selected because they require less space and less food and

normally grow faster than larger ones. After an initial quarantine and inanition period of two weeks they were placed in groups of three in 600 cc. of aged sea water. Each individual was marked with colored sealing wax and weighed bi-weekly. Three times each week enough washed bacteria were added to give 500 million per cc. of *B. marinus* or 5000 million *Rh. agilis*. The water was aerated in the dark at 14–15° C. and was changed once a month to obviate the accumulation of metabolic products in toxic concentrations. The mussels in 8 jars were fed exclusively with *Rh. agilis*, 8 others received *B. marinus*, and the mussels in the 8 control jars were not fed. The results are summarized in Table I.

TABLE I

AVERAGE WEIGHT OF MUSSELS AFTER BEING ON AN EXCLUSIVE DIET OF BACTERIA FOR DIFFERENT PERIODS OF TIME

Time	*Food received*	*Living specimens*	*Average weight*	*Mean gain in weight*
			gm.	*percent*
Initial	*Rh. agilis*	24	3.62	0
	B. marinus	24	3.65	0
	None	24	3.71	0
2 months	*Rh. agilis*	22	3.57	− 1.3
	B. marinus	21	3.72	1.9
	None	20	3.56	− 4.0
4 months	*Rh. agilis*	19	3.81	5.2
	B. marinus	21	3.86	5.8
	None	14	3.42	− 7.8
9 months	*Rh. agilis*	17	4.08	12.4
	B. marinus	21	4.04	9.7
	None	9	3.11	−16.3

The mussels fed on the red cocci gained an average of 12.4 per cent in weight in 9 months, those fed on the sporulating rods gained 9.7 per cent, and the fasting controls lost an average of 16.3 per cent. It is estimated that during the nine-month period covered by the experiment each mussel received at least 60 to 80 grams of bacteria or 6 to 8 grams of solid material, since the bacteria contain about 90 per cent water. This would indicate that between 5 and 10 per cent of the solid matter of the bacteria was assimilated by the mussels although it must be recognized that part of the weight gained by the mussels is calcareous shell substances taken from the sea water. Also numerous bacteria develop at the expense of the waste products of the mussels. However, probably much more than 10 per cent of the bacterial substance was assimilated because the gain in weight does not take into con-

sideration the energy which has been consumed by the mussels. Professor T. Kincaid at the University of Washington has maintained oysters for several months with nothing to eat except bacteria. The oysters appeared to develop normally and their glycogen content increased. Allen (1921) has emphasized the importance of nannoplankton, including bacteria, in the diet of freshwater mussels.

Although mussels have been maintained in the dark on an exclusive diet of bacteria for nearly two years, they do not thrive as well on such a diet as they do on the food they find in running sea water in the light. This may mean that the assorted dietary occurring in sea water is more nourishing than bacteria alone or that other conditions of the experiment were more favorable. There was no evidence that the bacteria themselves were injurious to the mussels in any way unless they were added in such great numbers that bacterial respiration vitiates the water. None of the 31 different species fed to mussels in concentrations ranging from 500 to 5000 million per cc. exhibited any toxic effects. In each case the mussels started to filter soon after the addition of the bacterial suspensions, freeing the water of the turbidity caused by the latter. Even the two acid-fast *Mycobacteria* and the *Actinomyces*-like rod were filtered from the water and many cells thereof were ingested although they were not digested. According to Fox (1936) the mussel is able to exercise a marked degree of discrimination with respect to the quality of food, selectively rejecting certain poisonous substances or closing its valves in their presence.

While the bacteria themselves are not injurious, the metabolites of rapidly reproducing cultures are quite toxic to the mussels. In the presence of seawater peptone-broth cultures containing less than a hundred thousand bacteria per cc., the valves of the mussels remained closed and the animals ultimately died. That the peptone itself was not the toxic ingredient was proved by feeding peptone to the mussels. It was tolerated in concentrations as high as 2.0 per cent until the growth of bacteria vitiated the water. Similarly glucose, glycerol and asparagine, all of which promote the growth of bacteria when added to sea water, were tolerated by the mussels until the concentration of bacterial metabolites became high enough to force the mussels to close their valves and eventually die.

Some mussels survived two weeks in sea water containing 2.0 per cent peptone, glucose, glycerol or asparagine when refrigerated (0 to 4° C.) to retard bacterial activity. Under these conditions the mussels were also inactive but revived even when frozen in ice if the temperature was slowly raised again. Mussels could be kept active in the above mentioned nutrients at normal water temperatures (ca. 15° C.) by adding the equivalent of one part of acriflavine to 10,000 parts of sea water which inhibits bacterial multiplication but is tolerated by the mussels, thereby proving that it is bacterial metabolites and not peptone, glucose, glycerol or asparagine which are toxic.

EXPERIMENTS WITH SAND-CRABS

The sand-crab, *Emerita analoga*, is a detritus-eating crustacean which inhabits shallow bottoms. It feeds by extending its feathery setose antennae from the sand in which it buries itself and collecting whatever particulate organic matter that may come along. It was selected for a series of bacterial feeding experiments because of its abundance in the region and its adaptability to laboratory conditions.

The bottoms of liter jars were covered with clean sea sand to a depth of 25–30 mm. and 500 cc. of aged sea water was added. Ten sand-crabs measuring 8–10 mm. in length were placed in each jar. Enough of a washed suspension of bacteria was added to give around 500 million cells per cc. When disturbed or when toxic substances were added to the sea water the sand-crabs remained completely buried. Otherwise they extended their antennae always reaching for food. This they commenced to do soon after the addition of the bacterial suspensions slowly clearing the water of its turbidity. The cultural and microscopic examination of the fore-gut of the animals one or two hours after receiving a bacterial meal showed that they were fairly gorged with the bacteria.

The sand-crabs removed the bacteria from the water much more slowly than the mussels and their ultimate filtering efficiency was not nearly so great. In six to eight hours they reduced the number of suspended bacteria from over 500 million to less than a million per cc., whereas in control jars lacking sand-crabs the water remained turbid for a few days with several million bacteria per cc. in suspension. It seems incredible that the filtering mechanism of the crabs is sufficiently fine to enable them to remove particles from water as small as bacteria (diameter ca. 1.0 μ) but they appear to be equipped for collecting the bacteria on the feathery antennae from which they can be scraped off into the crab's mouth. It is highly probable that the sand-crabs have a tendency to agglutinate or clump the bacteria because direct microscopic observations revealed the presence of many more clumps of aggregated bacteria in jars of water containing crabs than in the uninhabited control jars. Mr. Weldon M. Lewis who is studying the food habits of *Emerita* in this laboratory suggests that bacteria may be clumped by being enmeshed in the gills from which they are raked off when the crab cleans its gills. The crabs also unquestionably ingest large numbers of bacteria adhering to the sand grains.

The bacteria ingested by the crabs were rapidly digested. Bacteria in different stages of lysis were found in the fore-gut of crabs when examined soon after being fed. If, however, they were transferred to sea water sans bacteria after a bacterial meal, only sand grains were to be found in the gut half an hour later. Enzymes which dissolve the bacteria were also demonstrated in the digestive tracts of the crabs by dissecting these organs from large specimens and using a technique similar to that described above for

mussels. However, due to the difficulties attending the extraction of the enzyme in appreciable quantities it has been feasible to test the extract on only three different species of bacteria, all of which were digested.

Are the bacteria utilized as food by the sand-crabs? Enough *Rhodococcus agilis* to give about 5000 million per cc. were added semi-weekly to four jars each containing ten crabs. Another four jars received a like suspension of *Bacillus marinus* and four others received *Flavobacterium boreale* Lundestad. The latter is a Gram-negative non-motile rod 0.5 to 0.7 μ by 1.6 to 2.6 μ occurring singly and occasionally in pairs. It grows readily on sea water agar with the production of circular, glistening, bright yellow colonies. The crabs in four control jars were not fed. The water was aerated by bubbling air through it and was changed bi-weekly. Also at bi-weekly intervals the crabs were recovered from the sand by washing the latter through a large mesh sieve. The survivors were counted and after being washed entirely free of sand were weighed collectively in tared bottles.

Whenever the water remained or became turbid in any jar it was examined for dead crabs and the survivors were weighed and transferred to fresh sea water. The casts of the specimens which had moulted were recovered and counted. Table II summarizes the results of such an experiment of five months' duration.

TABLE II

NUMBER OF SAND-CRABS SURVIVING AFTER BEING FED ON BACTERIA FOR 22 WEEKS. THE NUMBER OF MOULTED CASTS AND THE AVERAGE WEIGHT OF THE LIVING SPECIMENS IS ALSO GIVEN AFTER DIFFERENT INTERVALS OF TIME

Time	*Food received*	*Living specimens*	*No. of casts*	*Average weight*
				gm.
Initial	*Rh. agilis*	40	0	2.6
	B. marinus	40	0	3.1
	Fl. boreale	40	0	2.5
	None	40	0	2.8
4 weeks	*Rh. agilis*	36	0	2.6
	B. marinus	31	2	3.0
	Fl. boreale	35	0	2.7
	None	32	0	2.7
10 weeks	*Rh. agilis*	27	3	2.5
	B. marinus	29	10	3.4
	Fl. boreale	31	7	2.8
	None	17	0	2.3
22 weeks	*Rh. agilis*	12	9	2.9
	B. marinus	14	19	3.4
	Fl. boreale	9	14	2.7
	None	0	0	—

The significance of the average weight of the sand-crabs is to be questioned, due to the inaccuracies of weighing the animals and also because many specimens perished in the course of the experiment. However, the weights given in Table II are more likely to be too small after the fourth week rather than too large because more small than large specimens survived. Moreover, the weight of moulted casts could legitimately be added to the average weights since they represent part of the organisms. Nevertheless in spite of these sources of error, the experiment shows that by giving them careful attention sand-crabs can be maintained longer when fed bacteria than without food. Furthermore, the moulted casts from the bacteria-fed animals is indicative of their development. It may be significant that the sand-crabs fed on the two larger rods, *B. marinus* (1.3 x 8.1 μ) and *Fl. boreale* (0.6 x 2.1 μ) developed better than those fed on the smaller coccus, *Rh. agilis* (0.8 x 1.0 μ).

The sand-crabs are more sensitive to large doses of bacteria than the mussels. However, there was no evidence of the bacteria themselves being toxic in moderate concentrations. Only when they were present in sufficient numbers to vitiate the water by their metabolism were the bacteria injurious to the sand-crabs. The sand-crabs soon died in the presence of as little as 0.2 per cent of the bacterial nutrients, peptone, glucose, glycerol or asparagine. Under these conditons they could not be kept alive by refrigeration or selective bacteriostatic substances. Concentrations of acriflavine which were tolerated by the mussels were lethal for the sand-crabs.

Like the mussels, the crabs tolerated living bacteria better than heat-killed ones, probably because other bacteria which are always associated with the animals multiply at the expense of the organic matter from the heat-killed bacteria. Baier (1935) reports that in moderate doses heat-killed bacteria are just as nutritious as live ones. However, he records that young bacterial cells are more nutritious than old ones.

EXPERIMENTS WITH GEPHYREAN WORMS

The sipunculid Gephyrean worm, *Dendrostroma zostericola,* is a hardy animal capable of being maintained in the laboratory for a long time (Peebles and Fox, 1933). Normally it lives burrowed in mud which it ingests for the organic matter content. It extrudes its tentacles from its burrow as if the animal were also collecting planktonic organisms from the water for ingestion. The feeding habits of *Dendrostroma* lead one to believe that the abundant bacterial flora in marine muds, as well as those in the water near the bottom or adhering to particulate matter, may play an important role in the nutrition of this animal.

Three healthy specimens measuring 3–6 cm. in length by 4 to 6 mm. in width at the greatest diameter when contracted were placed in each of

several liter jars together with 60 cm. of sand and 500 cc. of aged sea water. Some of these were fed the red coccus, *Rhodococcus agilis,* some the yellow rod, *Flavobacterium boreale,* and some were not fed. Shortly after receiving such a bacterial meal the oesophagus and descending arm of the intestine were noted upon dissection to be actually colored red and yellow, respectively. Microscopic examinations revealed an abundance of the two types of bacteria along with much sand. Confirming the observations of Peebles and Fox (1933) the control worms which received no food had ingested very few sand grains, and only by cultural procedures could any bacteria be demonstrated in the oesophagus. No bacteria were found in the intestines of the starved worms.* The lack of bacteria in the ascending arm of the intestine is regarded as evidence that the ingested bacteria were digested by the worms.

Contrary to expectations the worms failed to gain weight even after being fed on bacteria for 14 weeks although they appeared to be in good condition. However, the starved controls lost more weight than those which were fed, thereby indicating a nutritive property of the bacteria. It is possible that the bacteria-fed specimens failed to gain weight because they were mature adults, the quantity of food may have been insufficient, the bacteria may not be a complete food, or other conditions may have been such that the food could maintain the worms although the latter were unable to grow.

It has been shown by MacGinitie (1932) that the echinoid Gephyrean worm, *Urechis caupo,* lived well in sea water on an exclusive diet of bacteria, *Pseudomonas* sp. He (1937) points out that mucus plays an important role in entrapping bacteria and other plankton organisms which *Urechis* ingests. The former observation has been substantiated and elaborated upon somewhat.

Adult specimens of *Urechis caupo* were placed in U-tubes 20 cm. long in which they fit snugly when contracted (diameter 8 mm.). They were immersed in sea water in 2-liter battery jars. The water was aerated and kept in the dark. After the worms became acclimitized to these artificial burrows they commenced to force water through the tubes. In some cases bacteria were added to the sea water to give suspensions of 500 to 5000 million per cc. and in other cases heavier suspensions of bacteria were placed with a pipette directly in the inlet of the U-tube. In either case there was evidence of the bacteria being removed from the water by the worms.

Using the latter feeding technique two worms were fed every other day with 5 cc. of a concentrated suspension of *Rh. agilis,* two others received a like amount of *B. marinus* and two controls were starved. One of the

* Although no bacteria were added to the control jars, there were always several thousand mixed microflora which presumably grow at the expense of the metabolic waste products of the worms.

starved worms died after 12 days. Its surviving companion had lost much weight and died after 19 days. At this time the worms fed on *Rh. agilis* had lost a little weight, while those fed on *B. marinus* had gained slightly. All of the worms were killed on the 28th day when they were inadvertently fed on toluol-preserved suspensions of the bacteria. Up until this time the worms exhibited no toxic effects from large doses of bacteria and they appeared to be well nourished.

ABUNDANCE OF BACTERIA IN THE SEA

If the mussels, sand-crabs, and Gephyrean worms used in the foregoing experiments are at all representative of other animals, there seems to be little question that bacteria, if sufficiently abundant in the sea, may play an important role in the nutrition of marine animals.

According to literature summarized by Benecke (1933) sea water in the photosynthetic zone contains only a few to a hundred bacteria per cc. although occasionally several thousand or more bacteria occur per cc. The bacterial population is more sparse at greater depths until the bottom is reached. This is essentially in accord with the more recent observations of Waksman et al (1933) in the Atlantic Ocean, and of ZoBell and Feltham (1934) in the Pacific. However, these counts have been obtained using the conventional plating procedures which detect only a small percentage of the viable bacteria. Bere (1933) found direct microscopic counts of bacteria in lake water to be 20 to 335 times higher than plate counts, and Waksman and Carey (1935) report direct counts of bacteria in sea water to be about 200 times higher than plate counts.

Even if there are actually 1000 times as many bacteria in sea water as indicated by plate counts, they would constitute a rather inappreciable mass at any one time. Assuming the average size of marine bacteria to be 1 cu. μ and if sea water contained as many as 100,000 per cc., this would be equivalent to only 0.1 mgm. of bacteria per liter. However, the rapidity with which bacteria multiply tends to compensate for their extreme smallness, and regardless of how they are enumerated, the count is merely an expression of the dynamic balance between their rate of multiplication and the rate of destruction of bacteria. Thus if the bacteria reproduce only once every three hours (and many reproduce much more often than this) and if they are eaten by marine animals at the same rate, 0.8 mgm. of bacteria would be produced per liter per day. In a year a liter of water may yield nearly 3 gm. of bacteria, or approximately 200 mgm. of organic matter. If only a fraction of this amount of bacteria is formed per year, cumulatively it is of considerable importance in the food cycle in the sea, particularly when it is taken into consideration that sea water contains only about 10 mgm. of organic matter per liter.

Viewed from a different angle, it seems doubtful if bacteria constitute a very large part of the dietary of animals which feed in sea water. According to Harding (1937) the ciliate, *Glaucoma pyriformis*, eats around 13,000 bacteria per hour and it was semi-starved if the concentration of bacteria was less than 600 million per cc. Based on calculations by Baier (1935) an average-sized protozoan living exclusively on bacteria would have to fish through over 10,000,000 times its volume of water containing 100,000 bacteria per cc. in order to consume an amount of bacteria equal to its own volume! A mussel or sand-crab weighing 5 gm. would have to filter at least 50,000 liters of such water in order to consume an amount of bacteria equivalent to its own weight. Our experiments indicate that a dynamic population of several million bacteria per cc. is required to maintain these animals. From their studies on the food of pelagic copepods and the quantity of water filtered per day, Fuller and Clarke (1936) concluded that bacteria are insufficient in number to serve as an exclusive diet of these plankton crustacea.

In bottom deposits where bacteria are much more abundant than in the overlying water, they may constitute an appreciable part of the dietary of mud-feeders. According to the literature and original work summarized by ZoBell (1938) the bacterial population or marine mud as determined by plating procedures ranges from a few hundred to several million per gram of wet mud, the mean being in the order of magnitude of hundreds of thousands of bacteria per gram. The total bacterial population may actually be 10 to 100 times higher than this considering that plate counts detect only a small percentage of the viable bacteria.

Bacteria probably multiply much more rapidly in bottom deposits than in water since there is more organic matter available there and most other conditions are more suited to their reproduction. If the cells reproduce only once every six hours, it is estimated that under favorable conditions several milligrams of cell protoplasm may be synthesized per day by the bacteria in the topmost five centimeters of a square meter of marine sediment. While some of the bacterial cells may undergo autolysis, the majority of them are probably consumed as food by bottom-dwelling animals at about the same rate as they are formed. Cumulatively bacteria may supply 2 to 30 grams of particulate animal foodstuff per square meter per year in fertile bottoms. There may actually be enough bacteria in some bottom deposits to serve as the exclusive source of food for certain bottom-dwelling animals although the bacterial diet is perhaps supplemented to a large extent by other types of food.

There are numerous animals which live in and on the upper layers of slime or mud on the ocean floor. Sometimes the organic matter of this slime consists of 5 to 10 per cent by weight of bacteria. The majority of the slime-feeders whose habits have been described exert some discrimination in the selection of food, but many of them take in the entire substrate, digesting

the usable portion and ejecting the other. Baier (1935) includes in this category certain *Nematodes, Tubificidae, Chironomidae,* mussels, slime-inhabiting *Crustacea, Rotifera,* ciliates and flagellates. He also mentions a group of bacteria-devouring animals which graze on solid objects like shells, rocks, water plants and the like. Certain snails, ostracods, copepods, and amoebae are representatives of this group. According to ZoBell and Allen (1935), the slimy film scraped from the bottoms of ships contained a few million to several billion bacteria per gram and it consisted of 8 to 9 per cent by volume of bacteria. They (1933) found as many as a million bacteria attached per sq. cm. on glass slides after 24 hours' submergence in the sea, and the number increased progressively with time until the surface became obliterated with sedentary animals which presumably settled there to graze on the bacteria. This is in agreement with the studies on freshwater bacteria by Henrici (1933).

DISCUSSION

Although there may not be many animals equipped with filtering mechanisms sufficiently fine to catch individual suspended bacteria, most animals could catch them when the bacteria are clumped in aggregates of appreciable size. Esterly (1916) points out that certain copepods as well as ostracods form pellets of minute particles including bacteria which are readily ingested. A consideration of even greater importance is that most marine bacteria are periphytes or epiphytes which grow attached to particles of organic matter, plankton or other solid surfaces (ZoBell, 1936). Not infrequently there is a mass of bacteria attached to a particle of organic matter or a plankton organism which is greater than the mass of substrate itself. Consequently an inestimable quantity of bacteria are ingested by virtually all types of animals and the foregoing feeding experiments as well as the observations of others warrant the conclusion that at least some of the bacteria are assimilated as food.

Luck, Sheets and Thomas (1931) cite many references to show that protozoa are nourished by bacteria, as do Doflein and Reichenow (1928) for the *Ciliate.* These latter workers conclude that bacteria make up a great part of the food of ciliates. According to Kofoid (1933) the multifarious marine ciliates, *Tintinoinea,* feed upon bacteria. Some 200 different plankton organisms were found by Voroschilova and Dianova (1937) to feed on bacteria in the Caspian Sea where an increase in bacteria resulted in an increase in plankton. Waksman and Carey (1935) believe that protozoa, copepods and other bacteria-consuming organisms are primarily responsible for the paucity of bacteria in sea water. Naumann's studies (Baier, 1935) on the plankton crustacean, *Cladocera,* the copepod *Diaptomus* and certain *Rotifera,* reveal that these animals possess a filtering mechanism capable of catching bacteria which constitute their main nourishment. Stuart, Mc-

Pherson and Cooper (1931) found that live bacteria satisfy the food requirements of the micro-crustacean, *Moina macrocopa*, but that alls pecies of bacteria do not serve equally well as food. *Artemia* were observed by Bond (1933) to thrive on bacteria. That bacteria are consumed as food for marine sponges has been shown by Pourbaix (1932). After observing the development of frog tadpoles on an exclusive bacterial diet, Burke (1933) concludes that probably a wide variety of vertebrates as well as invertebrates utilize bacteria as food.

Regardless of whether one-tenth or only one-millionth part of the food of marine animals consists of bacteria, cumulatively it is of quantitative significance. It is now the concensus of opinion that, contrary to Pütter's theory, dissolved organic matter directly plays a very minor role (Krogh, 1931) in the nutrition of marine animals. Few, if any, animals have a mechanism for ingesting more than a casual amount of dissolved organic matter but when it is converted into particulate organic matter by bacteria (Krizenecky and Podhrasky, 1927) the latter may be readily ingested by many types of animals. Pütter (1924) himself has recognized this as shown by his observations on the bacterial nutrition of copepods.

Bacteria are of importance in food cycles not only by converting dissolved organic matter into a particulate and consequently a utilizable form but they also attack much solid organic matter unfit as food for other organisms such as, for example, cellulose and chitin, mineralizing part of it and synthesizing assimilable bacterial cell substance from the rest. It is for this reason that Baier (1935) writes that it is not the decomposing plant susbtance but rather the bacteria, the cause of decomposition, which nourish the animals consuming it.

Besides the heterotrophic bacteria which merely transform organic matter, there are autotrophic bacteria which synthesize cell substances from carbon dioxide, water and minerals deriving their energy either from sunlight or from the oxidation of hydrogen sulfide, sulfur, ammonium, nitrite, methane or ferrous iron. Little is known concerning the abundance of such photo- or chemo-synthetic autotrophs in the sea although the occurrence of several types has been reported including various kinds of sulfur bacteria (Bavendamm, 1924), iron-oxidizing bacteria (Baier, 1937) and nitrifying bacteria (Benecke, 1933). According to Benecke (1933) purple sulfur bacteria sometimes occur in the sea in quantities sufficient to impart a reddish coloration to the water. Below the photosynthetic zone chemosynthetic bacteria are perhaps the only organisms which contribute to the food cycle in the sea. Over ninety per cent of the ocean is below the photosynthetic zone.

SUMMARY

It has been demonstrated that the sea mussel, *Mytilus californianus*, ingests and digests bacteria. Specimens have been maintained on an exclusive diet of bacteria for several months during which time they gained in size and weight. Most marine bacteria are not injurious unless they are present in such great numbers that their metabolic products vitiate the water.

Similarly bacteria sustain the growth of the sand-crab, *Emerita analoga*, to a limited extent. This crustacean is more sensitive to large doses of bacteria than is the mussel and it is not as efficient as the mussel in removing bacteria from suspension.

The Gephyrean worms, *Dendrostroma zostericola* and *Urechis caupo*, were found to eat bacteria and to derive nourishment therefrom.

It is doubtful if bacteria are sufficiently abundant in sea water to constitute an appreciable item in the diet of marine animals, but indications are that cumulatively they play an important role in food cycles by synthesizing cell substances below the photosynthetic zone and by converting waste or dissolved organic matter into a form which can be utilized by animals as food. In marine bottom deposits and as a constituent of the slime on solid surfaces bacteria may be sufficiently abundant to nourish certain animals.

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STUDIES ON THE ISOLATION OF BACTERIA-FREE CULTURES OF MARINE PHYTOPLANKTON

By

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Physiological studies on phytoplankton such as nitrogen assimilation requires the use of pure cultures. It is a matter of relative ease to separate the various forms and cultivate them under artificial conditions but difficulty is frequently encountered in freeing the phytoplankton of contaminating bacteria.

For the isolation of phytoplankton from the sea Allen (1913) and Schreiber (1927) recommend the use of mineral enrichment media exposed to light which favors the multiplication of photosynthetic organisms but not of saprophytic bacteria. Pringsheim (1924) gives directions for separating the various species of algae or diatoms from the enrichment cultures either mechanically by picking single cells under a microscope with a loop or micro-pipette, or culturally on solid media. The latter method consists of inoculating agar or silica gel media impregnated with mineral salts with a loopful of the enrichment culture and exposing to light. If the inoculum is properly diluted discrete colonies will develop which can be isolated with an inoculating needle, transferred to tubes of media and examined microscopically or culturally for purity.

Freedom from other algae or diatoms can usually be ascertained by the direct microscopic examination of slide preparations but it is necessary to inoculate nutrient media to prove the absence of bacteria because the latter may escape visual detection. Due to their tendency to grow associated with phytoplankton as epiphytes or periphytes (ZoBell, 1937) bacteria will nearly always be present. The careful examination of a single diatom may reveal the presence of numerous tenaciously-attached bacteria. In order to free the phytoplankton of these contaminants, it may be necessary to sub-culture them several times seriatim on enrichment media. Although the mineral-enrichment media do not provide for the multiplication of saprophytic bacteria, a few bacteria will nearly always grow at the expense of the organic matter synthesized by the photosynthetic organisms. It is only

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because the phytoplankton multiply much faster than the bacteria in the enrichment media exposed to light that it is possible eventually to obtain bacteria-free cultures by serial transfers. One's chances for success are enhanced by picking the colonies as soon as they appear before the associated bacteria have had an opportunity to multiply at the expense of the diatoms or algae which die as the cultures become older.

In an attempt to simplify and improve the procedure of eliminating the bacteria from pure cultures of phytoplankton several different methods have been examined.

Luck, Sheets and Thomas (1931) have summarized the methods of obtaining bacteria-free cultures of protozoa. It was thought that some of these methods might be applicable to our problems. Washing the organisms in several changes of sterile water is impracticable because it was found that most of the bacteria are firmly attached to the diatoms which were examined. ZoBell and Allen (1933) found that about half of the bacteria in the sea are periphytes which attach so tenaciously to solid surfaces that they resist being washed off with running water. This periphytic characteristic of bacteria also invalidated the use of the cataphoretic method (Chapman, 1929) of freeing the diatoms of the bacteria although in an electrical field most of the unattached bacteria moved to the anode while the diatoms moved to the cathode. While one can attempt to select with the micromanipulator the single cells which appear to be bacteria-free, many attached bacteria escape detection from the mere microscopic examination of the diatoms.

Because of their photosynthetic mode of existence one may expect phytoplankton to be more resistant to radiations than bacteria. However, we have not been able to find any intensity of wave-lengths between 2760 and 4100 Å which were bactericidal but not injurious to the diatoms. Several enriched raw cultures from the sea as well as cultures of *Nitzschia closterium*, *N. bilobata*, *Navicula* sp. and *Chlorella* sp. have been tried. Shallow layers of water containing the organisms, as well as agar plates seeded with them, were exposed for different time intervals to varying intensities of radiations from a quartz mercury vapor lamp. In no instance did the phytoplankton survive intensities of ultra-violet radiations which were required to kill the bacteria. Rather broad ranges of frequencies were used, however, so it is possible that a selective action may be found if narrow radiation bands are used. Also it is possible that wave-lengths shorter than 2760 Å may be more efficacious. According to Duggar (1936) wave-lengths from 1900 to 2950 Å are more bactericidal than the longer ultra-violet radiations.

Various workers have obtained bacteria-free cultures of protozoa by sterilizing the cysts with heat or chemicals. We find that there are many bacteria in the sea which are more heat-tolerant than the diatoms, which rules out temperature as a means of ridding phytoplankton of contaminating

bacteria. The observations are summarized in Table I which shows the relative numbers of bacteria and phytoplankton which survived exposure to different temperatures. The heat resistance of the bacteria was tested by plating out on nutrient agar samples of sea water which had been held at the stated temperatures for ten minutes. The number of colonies which developed from the sample held at 22° C. (room temperature) was arbitrarily taken as 100 per cent and the others were calculated upon a basis of this. The temperature tolerance of "phytoplankton" was determined by heating those mentioned above, including one mixed enriched culture, to the stated temperature for ten minutes and then incubating them to see if they grew.

TABLE I

PER CENT OF MARINE BACTERIA AND PHYTOPLANKTON SURVIVING TEN MINUTES' EXPOSURE TO THE STATED TEMPERATURES

Temperature in °C	22°	30°	40°	50°	60°	80°
Bacteria (sea water flora)	100	74.1	27.6	5.7	3.5	1.8
Phytoplankton (five specimens)	100	60	20	0	0	0

In the search for selective bactericidal substances the following have been tried in various concentrations ranging from that which was high enough to kill the mixed marine bacterial flora to that which was sufficiently low to be tolerated by the bacteria for ten minutes at room temperature: Toluene, chloroform, sodium carbonate, hydrochloric acid, chloramine T, sodium perborate, potassium chlorate, mercurochrome, boric acid, thymol and certain aniline dyes. With the exception of toluene and chloroform which are not readily soluble in water, critical concentrations of nearly all of the chemicals were found which killed many bacteria and in which the phytoplankton survived, but some bacteria always survived in concentrations of the chemicals which are lethal for the phytoplankton. In view of the great tolerance of certain kinds of bacteria it seems doubtful if any bactericidal agent will be found which will kill all contaminating bacteria without being deleterious to the phytoplankton. The use of the highest concentrations of the germicides tolerated by the diatoms may somewhat enhance one's chances of obtaining bacteria-free cultures, but the advantages of such treatment are not sufficiently great to warrant the procedure.

In some of the foregoing experiments it was noted that bacteria were attenuated by treatment with sub-lethal concentrations of certain germicides. This suggested the possibility of finding a selective bacteriostatic substance which would restrict the multiplication of bacteria in concentrations in which the phytoplankton would grow. Certain aniline dies were tried, of which there are many as pointed out by Churchman (1923a), which suppress the reproductive mechanism of bacteria without otherwise injuring protoplasm.

In the preliminary tests concentrations of the dyes ranging from 1 : 1000 to 1 : 100,000 were added to appropriate media for bacteria and diatoms respectively. The nutrient medium for the bacteria consisted of 0.5 per cent Bacto-peptone and 0.3 per cent beef extract in sea water. Tubes containing 10 cc. each were inoculated with a mixed culture of marine bacteria and incubated in the dark. The enrichment medium for the diatoms consisted of aged sea water to which was added 0.10 per cent KNO_3 and 0.005 per cent each of $Na_2HPO_4.12H_2O$, $CaCl_2.6H_2O$, $Na_2SiO_3.9H_2O$ and $FeCl_3.6H_2O$. It was dispensed in 10 cc. quantities in test tubes, inoculated with a pure bacteria-free culture of *N. closterium*, and exposed to light. The relative amounts of growth, as indicated by turbidity, which occurred after different periods of incubation are recorded in Table II.

TABLE II

COMPARATIVE MULTIPLICATION OF MARINE BACTERIA (MIXED CULTURE) AND DIATOMS (*N. closterium*) IN MEDIA CONTAINING DIFFERENT CONCENTRATIONS OF DYES

Dye	*Concentration*	*Growth of bacteria after*			*Growth of diatoms after*		
		4 days	*7 days*	*14 days*	*4 days*	*7 days*	*14 days*
Crystal violet	1 : 10,000	–	–	–	–	–	–
Crystal violet	1 : 100,000	–	+	++	+	++	++++
Brilliant green	1 : 10,000	++	+++	+++	–	–	–
Brilliant green	1 : 100,000	++	++++	++++	+	++	++
Thionin	1 : 10,000	+	++	++++	–	–	–
Thionin	1 : 100,000	++	++++	++++	–	+	+++
Basic fushsin	1 : 1000	–	–	++	–	–	–
Basic fushsin	1 : 10,000	+	++	+++	–	+	++
Acriflavine	1 : 10,000	–	–	–	+	++	++++
Acriflavine	1 : 100,000	+	++	++	+	++	++++
Fluorescein	1 : 1000	–	+	++	–	–	–
Fluorescein	1 : 10,000	++	+++	++++	+	+	+++
None (control)		++	++++	++++	+	++	++++

Acriflavine was the only dye in the series which proved to be bacteriostatic in concentrations which did not interfere greatly with the growth of the diatoms. Crystal violet showed no selective action, being equally inimical to bacteria and diatoms. Brilliant green, thionin, basic fuchsin and fluorescein were more toxic for diatoms than for bacteria. Acriflavine was more bacteriostatic when the cultures were exposed to the light than when they were incubated in the dark probably due to the photodynamical activation of the dye.

Additional experiments were performed with acriflavine using concentrations of the dye ranging from 1 : 5000 to 1 : 20,000. Table III shows the comparative numbers of bacteria and diatoms which grew. The data on bacteria were obtained by plating samples of raw sea water with nutrient media containing the dye and counting the colonies which developed. The diatom data were obtained by inoculating sea water containing nutrient salts plus the dye with *N. closterium* and determining the amounts of growth after 12 days incubation in the light by centrifuging 5 cc. of the medium in Hopkins tubes. The amount of growth or the number of colonies which developed in the respective media lacking dye was arbitrarily taken as 100 and the others calculated on a percentage basis.

Only 13 per cent as many bacteria (mixed microflora indigenous to the sea) developed colonies on medium containing acriflavine in a concentration of 1 : 10,000 as on similar medium lacking the dye. The growth of the diatom, *N. closterium*, was inhibited very little by this concentration of the dye. Similarly *N. bilobata*, *Navicula* sp. and *Chlorella* sp. grew in the presence of one part of acriflavine in 10,000 of sea-water medium.

Several bacteria-free cultures of algae and phytoplankton have been isolated from enrichment cultures to which acriflavine had been added. Under these conditions the bacteria associated with the raw cultures multiplied very little or not at all, the dye being more bacteriostatic in the mineral enrichment media than in the organic-matter-rich media in which the results reported in Table III were obtained. The dye was used in the

TABLE III

Comparative Multiplication of Bacteria and Diatoms in Sea Water Media Containing Different Concentrations of Acriflavine

	Concentration of Acriflavine					
	1 : 5,000	*1 : 7,500*	*1 : 10,000*	*1 : 15,000*	*1 : 20,000*	*None*
Bacteria (sea water flora)	6	10	13	38	67	100
Diatoms (N. closterium)	20	54	89	93	96	100

initially inoculated mineral enrichment medium as well as in a similar medium solidified with agar upon which the enriched culture was streaked to obtain pure cultures of the phytoplankton. Most of the colonies picked from the first sub-culture on the solid medium proved to be bacteria-free. With less than ten per cent of the cultures it has been necessary to resort to serial sub-cultures on solid medium to eliminate contaminating bacteria.

Unfortunately there are a few acriflavine-fast bacteria from which the phytoplankton can be separated only by repeated dilution by successive

sub-cultures. It is possible that some other selective bacteriostatic substance could be used in combination with acriflavine to inhibit the multiplication of such acriflavine-fast strains of bacteria. Churchman (1929b) found that a mixture of acriflavine and gentian violet was much more bactericidal than either dye used alone. Additional dyes alone and in combination are being tried in an attempt further to simplify the purification of cultures of marine phytoplankton.

Although the procedure has not been applied to enough phytoplankton nor under enough different conditions to warrant definite conclusions, the results obtained in the foregoing experiments seemed to recommend the use of acriflavine in enrichment media to facilitate the isolation of bacteria-free cultures of certain diatoms. Whether or not the procedure is generally applicable to marine phytoplankton can be ascertained only by working on many more cultures under various conditions of cultivation. Experiments along this line are being continued.

SUMMARY

The methods of isolating bacteria-free cultures of phytoplankton are discussed.

Periphytic bacteria which are tenaciously attached to the phytoplankton invalidate washing, cataphoresis or the use of the micromanipulator for freeing the phytoplankton of bacteria.

Ultra-violet radiations of wave-lengths between 2760 and 4100 Å sufficiently intense to kill contaminating bacteria also killed the diatoms which were tested.

Several bactericidal substances were tried but they were all lethal for *N. closterium* and other diatoms in concentrations which killed bacteria.

Certain aniline dyes were found to have a selective genesistatic action. Acriflavine in a concentration of 1 : 10,000 inhibits the multiplication of most marine bacteria without appreciably effecting the reproduction of diatoms. Its use in enrichment media greatly facilitates the isolation of bacteria-free cultures of certain phytoplankton.

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PLANKTON STUDIES. I. A PRELIMINARY INVESTIGATION OF THE PLANKTON OF THE TORTUGAS REGION*

By

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This is the first of a series of papers dealing with the plankton of two regions—the Tortugas and Long Island Sound—which are both shallow water areas but are otherwise extremely diverse in their ecological characteristics. The work should not be regarded as a general survey of either place, since no attempt is made to determine the seasonal variation or horizontal distribution. It is intended rather as a study of the relationships of the plankton with certain of the basic environmental factors, which may be observed during a short period of time, and which may or may not have a general application.

The Dry Tortugas are a small group of islands located in the eastern part of the Gulf of Mexico (N. 24° 38′, 82° 52′ W.) near the edge of the continental shelf. It is a region of shallow water and extensive coral reef formation. Two stations were established. Station 1 was midway between Loggerhead Key and Garden Key in 19 m of water. Station 2, half a mile west of Loggerhead Key, and within the outer reefs, had a depth of 3 m. In addition to these stations, a series of samples was collected at the edge of the Gulf Stream twenty miles south of the Tortugas. The analyses are recorded in Tables IV to VI.

PHYSICAL CONDITIONS

Temperatures recorded during the collection of samples varied from 27.68 to 28.92° C. There is, however, a diurnal variation of the surface water of all protected areas, and temperatures as high as 29.90° were noted during the late afternoon.

Light transmission in the Gulf is ordinarily very high (Clark, 1938). In the Tortugas region the water is slightly turbid, and according to Taylor (1928) the transparency, measured with a Secchi disc, is 6.6 to 14.1 m, as compared with 20.9 to 36.4 m in the deeper water farther south. Nevertheless, there can be little doubt that the plankton received ample light for maximum growth to a depth of at least fifteen meters. In the surface

* This work was done at the Tortugas Laboratory of the Carnegie Institution of Washington with facilities provided by that organization.

layers the high light intensity may have been an inhibitory factor. This is especially true since the solar radiation was high and relatively constant. During the period of the investigation there were only two days when it was cloudy as much as half the day.

The water in the Tortugas region showed a pronounced drift to the westward. The currents were much stronger at Station 1 than at Station 2; the latter area, being largely protected by Loggerhead Key, formed a relatively quiet backwater. The plankton and chemical environment observed at Station 1 were therefore more nearly typical of oceanic conditions in this region, since they were more recently derived from the deeper water to the east.

THE STANDING CROP OF PLANKTON

Plankton collections were made by pouring four hundred liters of water through a net of No. 20 silk. Half of the sample was preserved for a count of the animals and for weighing, and the other half was used for a determination of plant pigments by the Harvey method (1934). From three to ten liters of water were filtered through a Whatman No. 2 paper for a measurement of the total plant pigments, and at Station 2 the chlorophyll was separated from the acetone extract and measured colorimetrically (Riley, 1938).

The total range of plant pigments in all analyses of surface water was 370 to 2430 Harvey units per m^3, with an average of 924. The values obtained at Station 2 were generally lower than at Station 1, and the average was 681. The net plankton constituted less than two per cent of the total, the mean quantity being 17 Harvey units per m^3.

At Station 1 there was some variation in the vertical distribution of plant pigments, occasionally amounting to as much as fifty per cent. No consistency was noted in the variations, the maximum appearing anywhere between the surface and 15 m. At the Gulf Stream station there was a gradual decrease from the surface to 90 m, and then at 150 m, in the region of the thermocline, they increased to more than twice the surface value. The indirect evidence of phosphate distribution would indicate that the plants were actively growing to a depth of 90 m, but this is of course a doubtful criterion.

The quantity of plankton was much less than that found in higher latitudes. The net plankton was approximately one per cent of the spring bloom conditions in the English Channel described by Harvey, Cooper, Lebour, and Russell in 1935. This is perhaps not a valid comparison, for the ratio of net to total plankton may be higher there. But the total chlorophyll at Station 2, averaging 0.33 mg. per m^3, was only about four per cent of the summer crop in Long Island Sound and two per cent of the largest bloom observed there so far. The mean number of animals at the Tortugas,

1740 per m^3, was only about two per cent of the quantity found in the Sound. This is a fair comparison, for the sizes are similar.

OXYGEN EXPERIMENTS

In order to obtain an estimate of plankton productivity under natural conditions, it is necessary to separate, either temporally or spatially, the processes of accumulation and degradation. Thus the ingenious method used by Atkins (1926) and by Harvey and his associates (1935) for measuring phytoplankton production in the English Channel is applicable only during the first half of a bloom, when the ratio of phosphate regeneration to phosphate consumption is negligibly small. In tropical shallow water areas there seems to be no possible method for making a natural estimate, so that it was necessary to resort to experimental means. The method adopted was the well known one of suspending in the sea light and dark bottles containing plankton, and subsequently measuring the oxygen production and consumption. In order to keep conditions as nearly natural as possible, the bottles were filled with ordinary sea water and suspended at the same depth from which the sample was taken. The period of immersion was five to seven days, for it was found that the oxygen production during shorter periods was not sufficient to counterbalance the normal errors of sampling and analysis. But in spite of the long duration of the experiments, it appears that the plankton remained in good condition. In some cases gallon bottles were used, and the plant pigments as well as the oxygen were measured at the end of the experiment. The phytoplankton lived and generally increased in the light bottles, and the decrease in the black bottles was slight.

Some error is introduced by absorption of light during its passage into the clear bottle; also, the black bottle has a slightly higher temperature. Further errors are caused by differences in oxygen consumption in the two bottles. These will be discussed in detail later. For the time being it is sufficient to say that the values recorded for oxygen production are not absolute, but since a large proportion of the errors are systematic in one sense or another, there is justification for regarding the experiments as indices of the potential photosynthetic activity of the various plankton associations studied.

The mean quantity of oxygen produced in all samples of surface water, measured by the Winkler method, was 1.31 mgs. per liter per week, and at Station 2 it was 0.92 mg. The total variation was very large, 0.21 to 5.36 mgs. There were, however, only two values of more than two milligrams per week. Neither is above suspicion because in both cases the results were due to the decrease of oxygen in the dark bottle and consequently may have been caused by the random inclusion of an unusually large amount of detritus. This is a serious problem when collections must be made near

coral reefs. At Station 1 the surface samples for both July 16 and July 26 may be at fault in this respect. On these days the maximum oxygen production was at the surface, with secondary maxima at 10 m. In the other two series at Station 1 the maximum was at 10 or 15 m. But in all cases the net increase of oxygen in the clear bottles was greatest at 10 or 15 m. If that is a good estimate of the depth of maximum photosynthesis, it is evident that it is considerably greater than in northern waters. In the deep water of the Gulf, where the transparency is greater, the difference would probably be still more marked.

NUTRIENTS

Soluble phosphate, determined by the Atkins-Denigès method, was found to be moderately low in all surface waters examined, the total variation being 0.4 to 3.2 mgs. per m^3, and the mean 1.3 mgs. No analyses were made of nitrate or iron.

In order to determine the limiting factors, bottles of sea water were enriched with nitrate, phosphate, or combinations of the two salts, in amounts varying from 10 to 100 mgs. per m^3. They were then suspended in the sea at a depth of one meter for a week, and at the end of that time they were analyzed for plant pigments and oxygen. The results of these experiments are shown in Table VII and in Figures 101 and 102.

In Experiment 2 additions of 10 and 20 mgs. of *P* per m^3 caused slight increases, especially in plant pigment production; otherwise the effect of adding phosphate appeared to be negligible. Addition of nitrate, however, produced strong positive effects in all but two of the experimental bottles. It was clearly much more important as a limiting factor than phosphate. Phosphate and nitrate together generally increased growth more than nitrate alone, but it is impossible to determine at what point the effect of phosphate became significant. In that respect the data for plant pigments and oxygen production are conflicting.

In Experiment 1, when iron was added along with the other two substances, there was a further increase in growth, but it was obviously not a very important factor, for considerable growth occurred without its addition.

DISCUSSION

In order to investigate the relationships between plankton and environmental factors, it is first necessary to obtain some sort of estimate of productivity. When Harvey (1934a and 1935) showed that the size of the standing crop of phytoplankton is largely controlled by animal consumption, the uselessness of gauging productivity by the size of the standing crop became immediately evident. This is the reason for the oxygen production experiments described above.

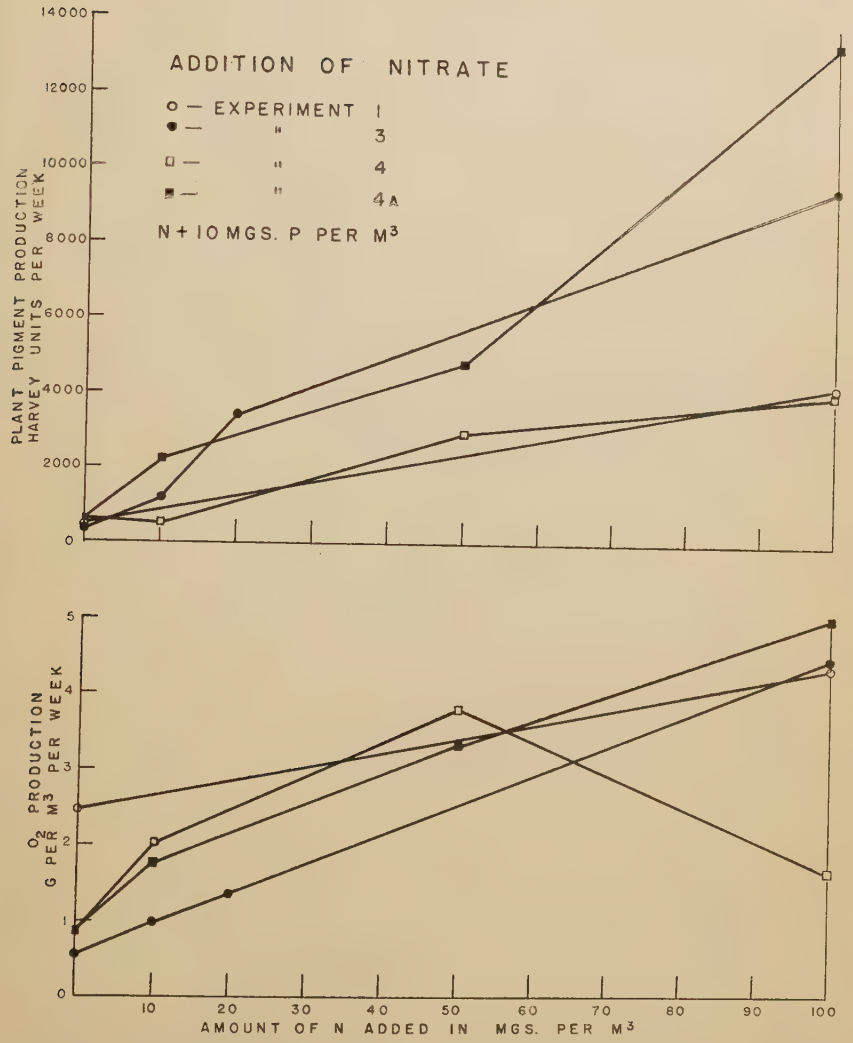

Figure 101. Effects of addition of Nitrate upon Plant Pigment production (*above*), and upon Oxygen production (*below*).

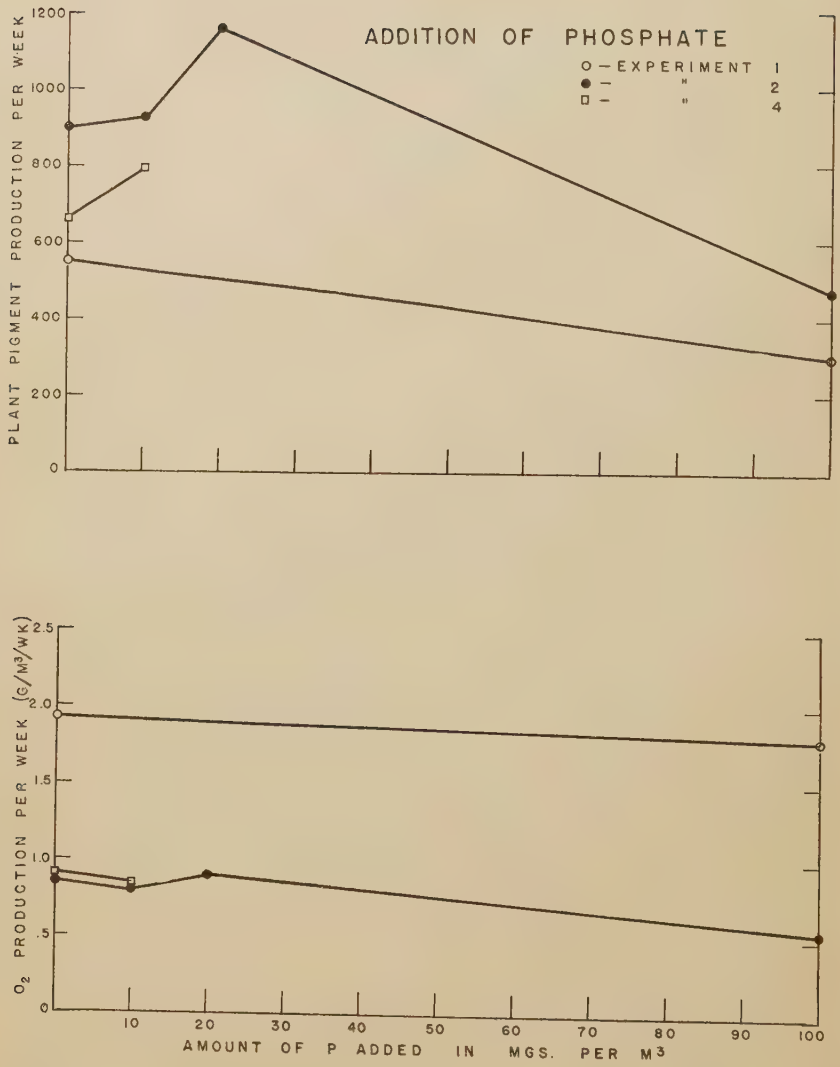

Figure 102. Effects of addition of Phosphate upon Plant Pigment production (*above*), and upon oxygen production (*below*).

It is reasonable to use photosynthetic activity as an index of the effectiveness of a given set of environmental conditions. It is particularly apt in this case because the relative constancy of temperature and light permit the assumption that photosynthesis is directly proportional to productivity.

The real question involved is whether or not the oxygen experiments can be used as an estimate of photosynthesis. It is a complex question which at present will be dealt with only from the theoretical standpoint.

In stating oxygen production as the difference between the oxygen content of light and dark bottles, the assumption is inherent that the oxygen consumption is the same in both bottles. This is not true because, in the first place, the mean quantity of phytoplankton is generally greater in the light bottle. A better value would be the sum of the observed oxygen production and the oxygen consumption of this additional quantity of phytoplankton. But unless differential feeding is unusually great, the increase of phytoplankton in the light bottle is proportional to the productivity. Therefore, as far as this error is concerned, the ratio between the observed and the absolute values for photosynthesis remains relatively constant.

In the second place, the respiration of animals is almost certainly greater in the clear bottle (Marshall, Nicholls, and Orr, 1935). Consequently, the presence of animals always diminishes the observed oxygen production, and the magnitude of the error is not related to the productivity.

Finally, as Jenkin suggests (1937), the respiration of phytoplankton may possibly be greater in the light than in the dark. Therefore in some cases she uses the increase of oxygen in the light bottle, the "net photosynthesis," rather than the total. In this case, however, it is not sound to do so because of the presence of animals and bacteria.

The experiments are therefore subject to three types of error, or perhaps two, depending on whether or not the third supposition is regarded as valid. These errors may or may not be large enough to be significant, but it is suspected that they are. They are systematic in the sense that they are related to the variables under consideration. The first is proportional to the oxygen production, the second to the quantity of animals, and the third to the phytoplankton. An attempt will be made in a subsequent paper to evaluate the errors. Without doing so it is possible to compare the relative effects of different factors on oxygen production, but the results apply only to experimental conditions, which differ from the normal reactions in sea water according to the magnitude of the errors involved. The only conclusion that can be drawn at present in regard to this difference is that the experiments give a minimal estimate of photosynthesis.

The difference between the plant pigments in the two bottles might be used as an estimate of productivity if it were not that somewhat similar errors are involved. The consumption of phytoplankton is probably greater in the light bottle because the animals are more active and also

because the larger quantity of phytoplankton makes it more available for consumption. The plant pigment production is therefore regarded as a minimal estimate of productivity.

The first step in the evaluation of the relationships of the different variables is the calculation of their coefficients of correlation. These are tabulated in Tables I and II, and the variations of plant pigments, chlorophyll, phosphate, animals, and oxygen production are shown in Figure 103.

TABLE I

Coefficients of Correlation Derived from Surface Analyses at Stations 1 and 2

	P	*O_2prod.*	*Animals*
Plant pigments	.430	.073	−.233
P		.366	.183
O_2 production			−.358

TABLE II

Coefficients of Correlation Derived from Analyses at Station 2

	Plant pigments	*P*	*O_2prod.*	*Animals*	*Organic matter*
Chlorophyll	.435	−.471	.404	−.499	−.456
Plant pigments		.158	.443	−.670	
P			.193	.181	−.026
O_2 production				−.551	−.224
Animals					.389

Most of the correlations in Table I are too low to be useful. This may be partly due to a lack of homogeneity between the results obtained at the two stations, but the errors discussed above in the analyses of oxygen production at Station 1 are also partly responsible. In further calculations it is therefore necessary to deal only with Station 2, an unfortunate situation because it leaves only nine complete sets of data, not enough to permit a very exact analysis.

It would appear from the correlations that the chief factors affecting oyxgen production are the standing crop of phytoplankton and the quantity of animals. The relationships with phosphate and organic matter are not very significant. The negative correlation of organic matter with oxygen production is presumably caused by the animals. Theoretically, the phytoplankton and detritus should have a positive effect, the latter of course because of liberation of nutrients during decomposition. But when the weight of animals, estimated by means of a regression equation, is deducted from the total, the correlation between the residual organic matter and oxygen production is only .080, so that the positive effect is probably insignificant.

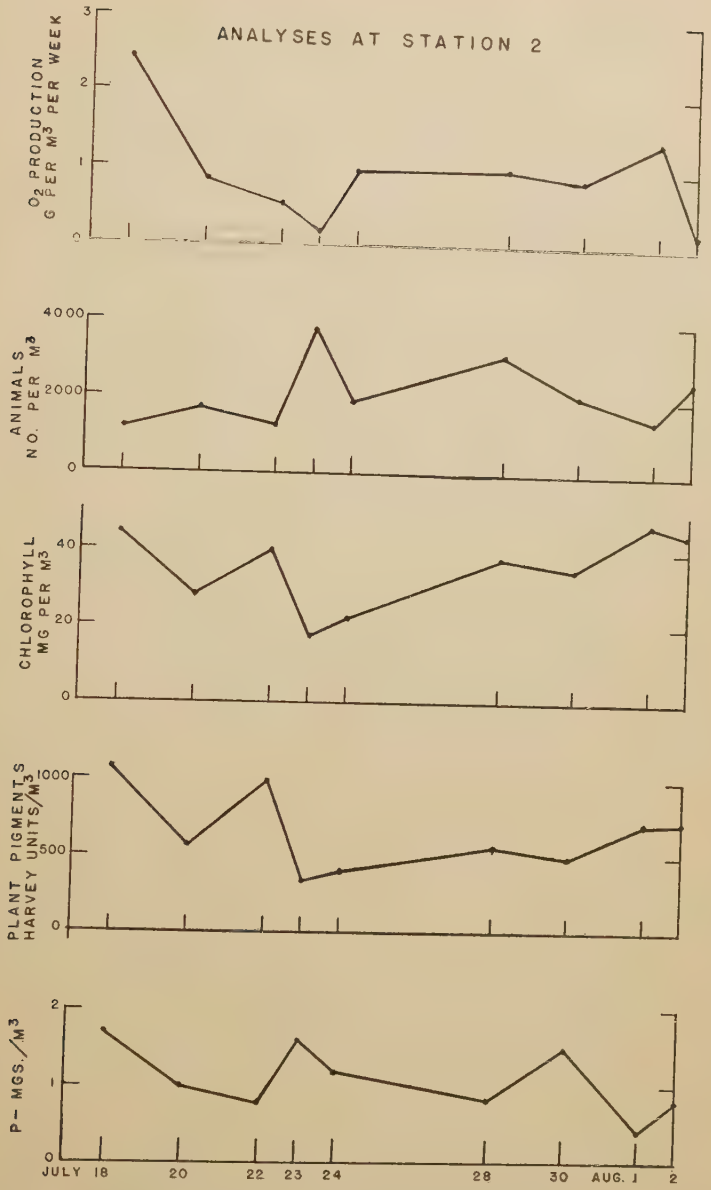

Figure 103. Variations in oxygen production, number of animals, chlorophyll, plant pigment, and phosphate at station 2.

The relative importance of different factors is best determined by means of multiple correlations. The multiple regression equation used here (cf. Bruce and Reineke, 1931) has the form

$$W = \overline{W} + Bwx\frac{\sigma w}{\sigma x}(X - \overline{X}) + Bwy\frac{\sigma w}{\sigma y}(Y - \overline{Y}) \qquad (1)$$

where W is the dependent variable, X and Y are the independent variables, $\overline{W}$, $\overline{X}$, and $\overline{Y}$ are the means of the variables and σw, etc. their standard deviations, and Bwx and Bwy are constants calculated from the intercorrelations by the least squares method.

The coefficients of correlation used in the following equations have been presented in Table II. The means and standard deviations are shown in Table III.

TABLE III

MEANS AND STANDARD DEVIATIONS DERIVED FROM THE ANALYSES AT STATION 2*

	Mean	σ
Plant pigments	.660	.236
Chlorophyll	.351	.097
P	1.09	.415
Animals	2138	824
Organic matter	11.44	3.71
Detritus	5.83	3.51
Oxygen production	.960	.671

Utilizing the two variables which appear to have the greatest effect on the quantity of oxygen produced, chlorophyll and animals, the equation is

$$W = 1.19X - .00038Z + 1.35 \qquad (2)$$

where W is O_2 production in g. per m^3 per week, X is chlorophyll in mgs. per m^3, and Z is the number of animals per m^3. The correlation between the observed and calculated values for oxygen production is .550. This is a slight improvement over the simple correlation between chlorophyll and oxygen production, but considering the small quantity of data used, the result is hardly satisfactory.

The next step is to examine the effect of minor factors. When phosphate is included, the equation becomes

$$W = 2.81X + .75Y - .00035Z - .09 \qquad (3)$$

* The analyses of July 19 are omitted because there are no data on animals and organic matter for that day. For this reason the mean values given here for plant pigments, chlorophyll, oxygen production, and phosphate are slightly different from those listed in the descriptive sections.

where Y is phosphorus in mgs. per m^3, and the symbols for the other variables are the same as in Equation 2. The correlation between observed and calculated values for W is .706, a considerable improvement.

The question then arises as to the effect of detritus. The method of obtaining this value should be explained more in detail since it is a useful tool for rough estimates of this sort. Part of the variations in the weight of organic matter are dependent on variations in the quantity of animals, as shown by the correlation of .389. The relation between the two may be expressed according to the product moments formula

$$Y = \overline{Y} + \frac{\Sigma dx dy}{\Sigma d^2 x} (X - \overline{X}) \tag{4}$$

where X is the independent and Y the dependent variable, $\overline{Y}$ and $\overline{X}$ are the means, and dx and dy the deviations from the means. When applied to the present data, the equation becomes

$$Y = .00263X + 5.80 \tag{5}$$

where Y is the weight of organic matter in mgs. per m^3 and X is the number of animals per m^3. It is therefore possible to compute the weight of animals for each date and obtain the residual organic matter by difference. The values so determined are accurate provided the relative size of the animals remains constant, and the amount of detritus bears no relation to the quantity of animals. The first assumption is reasonably correct in this case; the second may possibly be subject to error, in which case the calculated weight of animals would be slightly too high.

Using the values so obtained, the equation for oxygen production is

$$W = 3.39X + .82Y - .00030Z + .03Z_1 - .67 \tag{6}$$

where Z_1 is the estimated weight of detritus in mgs. per m^3, and the other symbols are as given above. The final correlation is .701, approximately the same as the result obtained in 3. The effect of variations in organic matter is therefore regarded as negligible, and Equation 3 is accepted as the most satisfactory statement that can be derived from the available data. The calculated effect of these factors on oxygen production is shown in Figure 104.

Because of the small size of the sample, some question may arise as to just what the results mean. Using the method described by Fisher (1930) for computing the probable error, it is found that in the case of the simple correlations between chlorophyll and oxygen production and between animals and oxygen production, the probability that the observed results are due only to chance is about 1 : 10. This of course does not satisfy rigid statistical requirements. But since the results fit together neatly, forming

a multiple correlation with a probability of 1 : 50, there is not much doubt that the method is valid and the observed effects of chlorophyll and animals, and probably of phosphate, are real. Final proof will of course depend on the results of subsequent analyses.

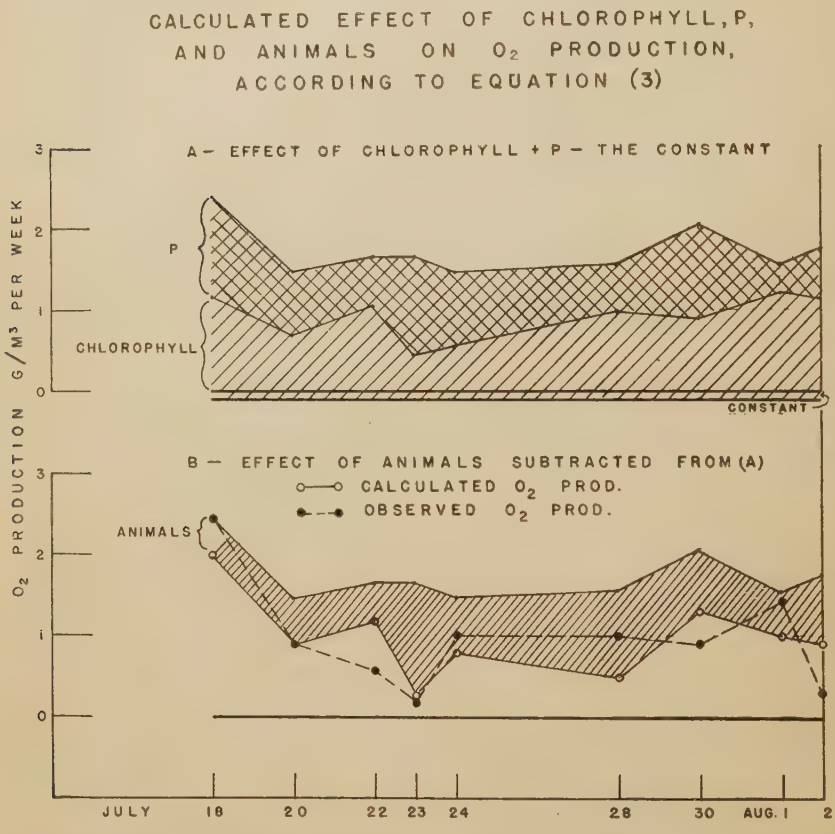

Figure 104. Effect of chlorophyll, phosphate, and animals on oxygen production, calculated according to equation (3), compared with observed oxygen production.

The proper interpretation of a multiple regression equation requires some care. We are interested in the relative effect of a given factor on oxygen production, and especially in determining, within certain limits of error, what the oxygen production should be with a given set of independent variables. The accuracy with which this can be done is somewhat limited by the nature of the equation. The effect of factors not listed as independent

variables is accounted for by the final constant. Thus it is mathematically assumed that all the other factors are constant in their effect, and when this is not true the result appears as a difference between the observed and calculated values of the dependent variable. This much is simple and to be expected. But the balance may be upset by relations between independent variables, so that bringing another factor into the equation may change one or more of the constants. When, as in the case of phosphate and animals, the constant remains relatively unchanged in two or three equations, it is justifiable to regard it as reasonably accurate. But the chlorophyll presents a different problem, since the addition of each independent variable changes its relative effect. It is evident, therefore, that the chlorophyll function is related to the constant, and the relative effect of the standing crop cannot be evaluated accurately as long as any significant factor remains undescribed. It is reasonable to suppose, both from the biological and the statistical evidence, that the chlorophyll may serve not as a true independent variable but rather as a function of all the other variables.

In estimating the relative effects of phosphate and animals, the standard deviation is a convenient yardstick. According to Equation 3, a "normal" variation in phosphate, that is, a change from the mean to the limit of its standard deviation, changes the oxygen production .311 g. per m^3 per week, provided all other factors remain constant. This is 38% of the maximum change produced by shifting all the independent variables to the limits of their standard deviations. The effect of animals, determined in the same way, is .288 g., or 36%. It is 46% and 43%, respectively, of the standard deviation of oxygen production, which is biologically a more sound value for comparison, although it does not give a true ratio for all factors involved.

Harvey and his associates have shown that consumption is an important factor in controlling the size of the standing crop. The evidence presented here is a direct confirmation since it is shown that they have a negative effect on both chlorophyll and oxygen production. There is every reason to believe, however, that the latter effect is only a temporary phenomenon. Over a long period of time, rapid consumption should increase the rate of turnover of nutrients, because not only would the metabolism of living animals speed up regeneration, but also the decomposition of zooplankton would probably be faster than that of phytoplankton (Cooper, 1935). This effect would be especially marked in shallow areas but might occur to some extent in deep water.

The effect of phosphate is problematical, for the experiments and the statistical analysis are in contradiction. There are several possible explanations:

1. The statistical treatment may be faulty in this respect.
2. Phosphate may have some effect as a limiting factor in the strict sense. There is evidence for this in Experiment 2.

3. Limiting factors are not always absolute in their action. Unpublished experiments on freshwater plankton by G. E. Hutchinson and the present author have shown that an increase of a particular nutrient will sometimes cause a slight stimulation of growth even though it is not a true limiting factor. Such a situation may possibly be operative here.

4. It is possible that variations of nitrate and phosphate are related and that phosphate has no significance in the equation except as an indicator of variations in nitrate. Such a relationship is the normal result of previous plankton growth and is a well known phenomenon (Harvey, 1928; Cooper, 1937; and others).

There is no evidence that permits acceptance of one of these possibilities in preference to any of the others. Considering all the facts at hand, it is possible only to say that phosphate, while it may be a limiting factor in the strict sense, is not as important in this respect as nitrate; nevertheless, its variations are related to plant utilization, as shown by the negative correlation between chlorophyll and P, as well as the more doubtful positive relation with oxygen production.

In all the equations chlorophyll has been used as the index of the phytoplankton crop. It cannot be used interchangeably with the values for plant pigments without considerable error because the relation between the two is unfortunately not very close. This is due to differences in the ratio of carotinoids to chlorophyll. Measurements of the carotinoids were made at Station 2 with an arbitrary standard of potassium dichromate. They are not listed here because they serve no useful purpose other than in this particular matter, but they show clearly that the differences in question are real and are not caused by analytical errors. The correlations show no evidence that the results are influenced by the carotinoid pigments of animals; the variations appear to be within the plants themselves.

From the dynamic standpoint of relationships with oxygen production, the chlorophyll method is theoretically superior. This advantage is largely offset by the simplicity of the method of determining plant pigments. It can be used in circumstances that make the chlorophyll measurement virtually impossible. The simple correlations are of about equal magnitude in either case. The multiple correlation reveals some differences. When, in Equation 3, plant pigments are substituted for chlorophyll as the value for X, it is changed to

$$W = 2.44X + .43Y - .00038Z - .30 \qquad (7)$$

The final correlation is .585, which is not a significant improvement over the simple correlation.

ACKNOWLEDGMENTS

I take great pleasure in thanking The Carnegie Institution of Washington for permission to work at the Tortugas Laboratory and for their kind co-operation in providing me with the necessary materials for the investigation. I am especially indebted to Dr. Tennent and Dr. Conger, and to Captain Mills and his staff, all of whom aided me in every possible way.

SUMMARY

1. The Tortugas region is essentially a tropical environment, with high and relatively constant light and temperature and moderately low nutrient content.

2. The quantity of plankton was very small, less than four per cent of the amount ordinarily found in similarly shallow areas in temperate regions.

3. Nitrate was the most important limiting factor. The effect of phosphate was slight but probably significant.

4. The photosynthetic activity of the plankton was studied by measuring oxygen production and consumption in clear and blackened bottles. It was found that the depth of maximum photosynthesis lay between 10 and 15 meters.

5. Multiple correlations are used to evaluate the relative importance of phosphate and the size of the standing crops of plants and animals in respect to their effect on the photosynthesis of the phytoplankton.

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TABLE IV

ANALYSES* AT STATION 1

	July 16, 1938				*July 21, 1938*			
Depth	1	5	10	15	1	5	10	15
Temperature	28.54	28.55	28.51	28.50	28.58	28.58	28.58	28.58
Oxygen	6.41	6.42	6.40	6.18	6.46	6.41	6.41	6.44
P	3.2	3.4	3.9	4.3	0.6	1.6	2.9	1.6
Plant pigments	2430	2230	2090	2040	1530			
O_2 production	1.57	0.99	1.15	0.70	0.43	0.36	1.25	1.72
O_2 consumption	1.49	0.74	0.71	0.43	0.86	0.48	0.64	0.83
Net plankton:								
Plant pigments	55				10			
Organic matter	30.0				7.0			
Animals	800				1260			
	July 26, 1938				*July 31, 1938*			
Depth	1	5	10	15	1	5	10	15
Temperature	28.21	28.18	28.11	28.05	28.33	28.31	28.30	28.10
Oxygen	6.35	6.44	6.55	6.52	6.05	6.10	6.12	6.15
P	2.2	2.2	1.8	1.8	0.9	0.7	1.3	1.7
Plant pigments	780	830	1200	1080	1330	1570	1130	2640
O_2 production	5.36	1.05	1.13	0.76	0.92	0.91	0.74	0.99
O_2 consumption	4.75	0.48	0.41	0.36	0.52	0.27	0.20	0.14
Net plankton:								
Plant pigments	7				19			
Organic matter	11.5				10.5			
Animals	380				990			

* Note: The analyses are recorded as follows: Temperature, °C.; Oxygen, mgs. per liter: P, mgs. per m^3; Plant pigments, Harvey units per m^3; Chlorophyll, mgs. per m^3; Oxygen production and consumption, mgs. per liter per week; Organic matter, dry weight in mgs. per m^3; Animals, number per m^3; Depth, meters.

TABLE V

ANALYSES AT STATION 2

Date	*July 18*	*July 19*	*July 20*	*July 22*	*July 23*	*July 24*	*July 28*	*July 30*	*Aug. 1*	*Aug. 2*
Temperature	28.51	28.92	28.16	28.21	27.93	27.68	28.62	28.26	28.23	28.51
Oxygen	6.38	6.25	6.02	6.10	6.09	5.49	6.09	6.44	5.99	6.31
P	1.7	2.7	1.0	0.8	1.6	1.2	0.8	1.5	0.4	0.8
Plant pigments	1110	870	570	980	370	410	560	500	700	740
Chlorophyll	0.44	0.15	0.28	0.40	0.18	0.22	0.38	0.35	0.47	0.44
O_2 production	2.43	0.60	0.86	0.53	0.21	0.98	1.08	0.90	1.43	0.22
O_2 consumption	2.48	1.11	1.13	0.87	0.45	0.83	0.59	0.63	0.60	0.59
Net plankton:										
Plant pigments	25		17	9	12	8	21	15	11	18
Organic matter	7.5		15.0	6.0	10.5	16.5	16.0	11.5	7.5	13.0
Animals	1210		1690	1310	3850	1900	3180	2080	1520	2500

TABLE VI

ANALYSES AT GULF STREAM STATION, 20 MILES SOUTH OF LOGGERHEAD KEY. DEPTH 166 METERS. AUGUST 6, 1938

Depth	1	18	45	90	153
Temperature	28.34	27.94	23.55	21.40	11.59
Oxygen	6.38	6.42	6.50	6.46	4.38
P	2.9	0.4	3.1	3.1	28.8
Plant pigments	600	630	520	410	1240
Net plankton:					
Plant pigments	8				
Organic matter	57.0				

TABLE VII

EXPERIMENTS

	Plant Pigments	*Oxygen*	*Oxygen Production*	*Pigment Production*
Experiment 1 July 18–25, 1938				
Initial values	1110	6.38		
Control	1050	6.33	2.43	540
Dark bottle	510	3.90		
N (100 mgs/m³)	4750	7.24	4.34	4240
P (100 mgs/m³)	810	6.20	2.30	300
N + P + Fe (100 mgs.)	3950	10.40	6.50	3440
N + P (100 mgs.)	2720	9.88	5.98	2210
Experiment 2 July 20–27, 1938				
Initial values	570	6.02		
Control	1470	5.75	0.86	900
Dark bottle	570	4.89		
P (10 mgs./m³)	1500	5.72	0.83	930
P (20 mgs./m³)	1760	5.81	0.92	1190
P (100 mgs./m³)	1040	5.42	0.53	470
Experiment 3 July 22–29, 1938				
Initial values	980	6.10		
Control	1400	5.76	0.53	410
Dark bottle	990	5.23		
N (10 mgs./m³)	2190	6.23	1.00	1200
N (20 mgs./m³)	4440	6.61	1.38	3450
N (100 mgs./m³)	10590	9.68	4.45	9600
Experiment 4 July 30–August 4, 1938				
Initial values	500	6.44		
Control	1000	6.63	0.90	660
Dark bottle No. 1	460	5.93		
Dark bottle No. 2	600	6.05		
P (10 mgs.)	1100	6.62	0.88	800
N (10 mgs.)	930	7.43	2.02	720
N (50)	2600	8.72	3.82	2900
N (100)	3450	7.14	1.61	4090
N (10) + P (10)	1930	7.22	1.72	2220
N (50) + P (10)	3870	8.34	3.29	4680
N (100) + P (10)	10130	9.57	5.01	13440

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